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1958 Iodine Research Award Nominations Requested Prior to January 1, 1958

Nominations are now being received by the AMERICAN PHARMACEUTICAL ASSOCIATION for the 1958 Chilean Iodine Educational Bureau, Inc., Award recognizing outstanding research in the chemistry and pharmacy of iodine and its compounds as applied in pharmacy or medicine.

The award consists of $1,000 and a diploma setting forth the reasons for selection of the recipient. It may be presented each year at the annual meeting of the ASSOCIATION.

Any member of the ASSOCIATION may propose a nominee by submitting eight copies of each of the publications to be considered in the competition, a biographical sketch of the nominee including date of birth, and a list of his publications. Eight copies of the nomination must be submitted to Robert P. Fischelis, Secretary of the AMERICAN PHARMACEUTICAL ASSOCIATION, 2215 Constitution Ave., N. W., Washington 7, D. C. To be eligible for the 1958 Award, *nominations must be received on or before January 1, 1958.*

A nominee must be a resident of the United States or Canada. He must have accomplished outstanding research in the chemistry or pharmacy of iodine and its compounds as applied in pharmacy or medicine.

During the period covered by the nomination the nominee shall have been actively engaged in, shall have completed, or shall have published a report upon the line of investigation for which the award is made. During the period of two years prior to the date of nomination, the nominee shall not have been engaged in research under the sponsorship of the Chilean Iodine Educational Bureau, Inc.

The recipient will be selected by an award committee which is appointed by the chairman of the ASSOCIATION'S Council. The present committee includes Justin L. Powers, chairman; F. F. Blicke, E. A. Brecht, John E. Christian, Stanley G. Mittelstaedt, Lloyd M. Parks, and Linwood F. Tice.

The recipient will deliver a paper or lecture upon the subject of his scientific work at the meeting at which the award is conferred. His paper, or address, will then be published in the JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION. Travel expense to the meeting at which the award is to be made is provided for up to $250.

The award is now in its tenth year. The recipient of the first award was Dr. William T. Salter, Yale University pharmacologist; the second recipient was Dr. George M. Curtis, Chairman of the Department of Surgical Research and Professor of Surgery at Ohio State University, and the third was Dr. C. P. Leblond, Professor of Anatomy, McGill University, Montreal, Canada. The fourth award was shared jointly by Dr. George Moore of the University of Minnesota Medical School and Dr. Moses Ashkenazy, Chief of Neurosurgery, Veterans Hospital, Houston, Tex. Dr. Vernon H. Wallingford, Mallinckrodt Chemical Works, St. Louis, Missouri, was the recipient of the fifth award. Dr. Jack Gross, Associate Professor of Anatomy, State University of New York, College of Medicine in Brooklyn, received the sixth award and Dr. Dominick Papa, Schering Corporation, Bloomfield, N. J., received the seventh award. The eighth award went to Dr. John E. Christian, Professor of Pharmaceutical Chemistry, School of Pharmacy, Purdue University, Lafayette, Indiana. The ninth award was conferred on Dr. James O. Hoppe, Research Pharmacologist, Sterling-Winthrop Research Institute, Rensselear, New York, during the 1957 Convention of the AMERICAN PHARMACEUTICAL ASSOCIATION.

NOTICE

PAPERS FOR SECTION MEETINGS OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

The next annual meeting of the American Pharmaceutical Association will be held in Los Angeles, California, April 20 to 26, 1958. Titles and abstracts (not to exceed 200 words) of papers must be submitted to the appropriate Section secretary not later than February 1, 1958, or preferably earlier, in order to assure a place on any Section program.

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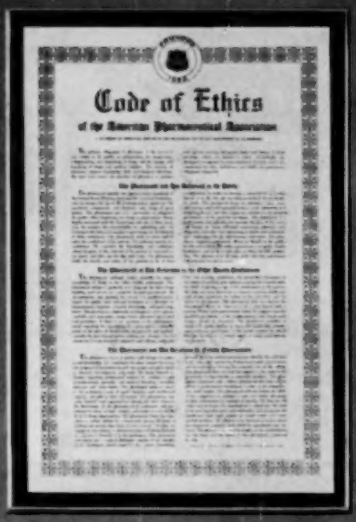

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VOLUME XLVI — *NOVEMBER 1957* — NUMBER 11

CONSECUTIVE NO. 22

Some Applications of an Integrating Sphere Reflectometer to Pharmaceutical Problems*

By C. W. McKEEHAN† and J. E. CHRISTIAN

An integrating sphere reflectance attachment was built for the Beckman DU spectrophotometer. This apparatus was then applied to the problem of determining color stability in a bentonite base cream. Rate constants for the decrease in reflectance of the cream aged at room temperature, 55°, 60°, 65°, 70°, and 75° were determined. A theoretical rate constant at 25° was found by use of the Arrhenius relation of reaction rate to absolute temperature. This value was used to predict color stability at 25°. Reflectance measurements were also made on coated tablets and powders.

ALTHOUGH MEASUREMENTS of reflectance have been used for one purpose or another for many years, their applications to pharmacy and its allied areas have been very limited. Some of the more recent applications of reflectometry to areas closely related to pharmacy include the grading of certain foods (1, 2), measuring the reflectance of human skin (3–5), and applications to analytical chemistry (6, 7). Theoretical aspects of reflectometers and reflectometry are readily available in the literature (8–10).

Recently, Garrett and Carper (11) applied the methods of absorption spectrophotometry and chemical kinetics to predicting the color stability of a sulfa suspension. Since the reflectometer may be used to measure the color of opaque solids and semisolids, the possibility of employing similar methods for the investigation of color stability in this type of pharmaceutical preparation is obvious.

The purpose of this project was: to design and evaluate the performance of an integrating sphere reflectance attachment for the Beckman DU spectrophotometer; to develop a method for predicting the color stability of ointments; and to show other possible applications of reflectometry to pharmacy.

REPRESENTATIVE APPLICATIONS OF REFLECTOMETRY TO PHARMACEUTICAL RESEARCH

Apparatus.—An integrating sphere reflectance attachment for the Beckman DU spectrophotometer is described in the following paragraphs. Basically, it is of the same design as the reflectance attachment for the Beckman DR spectrophotometer reported by Jacquez, *et al.* (12). Certain modifications were necessary because the DU is manually operated, whereas the DR is automatic recording. Instead of using the round phototube aperture described in (12), a $1^1/_4$ x $^3/_4$-in. rectangular opening was cut in the side of the sphere so that the standard

* Received May 3, 1957, from the School of Pharmacy, Purdue University, Lafayette, Ind.
Presented to the Scientific Section, A. PH. A., New York meeting, April 1957.
† Present address: Eli Lilly and Company, Indianapolis, Ind.

Beckman DU phototube housing could be used. The automatic beam-switching mirror was replaced with one which could be manually operated. A diffusing screen made from a photoflash diffusing shield was inserted between the sphere and the phototube housing to provide more uniform illumination of the phototubes. The angle of incidence of the light beam on the sample and reference was 18° instead of 16° 45′. To facilitate reflectance measurements on pharmaceutical semisolids and powders, the attachment was mounted so that one sample aperture would be horizontal. The sphere was machined as two $2^1/_2$-in. radius hemispheres from aluminum bar stock.

In order to increase sensitivity of measurement, a 10,000 megohm load resistor[1] was used. The inside of the sphere was coated with a 2-mm. layer of magnesium oxide to increase its reflection properties. Magnesium oxide was used because it is the most highly reflecting substance known (13). It was deposited on the inner surface of the sphere by burning short strips of magnesium ribbon and allowing the oxide to collect on the aluminum surface. Details of the coating procedure may be found in National Bureau of Standards Letter Circular LC-547 (14).

Jacquez, *et al.* (12, 15), have published papers on the theory and operation of this type of instrument. They have tested its performance in a number of different ways and report that in regions of intermediate or high reflectance, the instrument is capable of yielding reflectance values with less than 1% error. Methods similar to those described in these papers were used in evaluating the performance of the attachment reported here. The collecting efficiency[2] for the sphere coated with 2 mm. of MgO was found to be about 0.26 at 600 mμ when a MgO reference and a sample of reflectance 0.5 were used. Error in measurement due to the finite size of the apertures and use of flat, instead of spherical samples, was found to be approximately 0.007 for a reference of reflectance unity and a sample of reflectance 0.5. The error due to unsymmetrical positioning of the reference and sample apertures with respect to the phototube aperture was less than 1% throughout the region from 420 to 800 mμ. Effective band width was found to vary from 4.48 to 4.74 mμ for the same range. The upper limits for stray light were approximately 0.005 in this region.

Color Stability of a Cream.—Resorsul cream[3] was used as a representative preparation. Preliminary experiments indicated that a color change could be detected by storing the cream at elevated temperatures. Figure 1 shows the change in reflectance (R) of a sample aged at 65°. Plots of log R *vs.* time at 20 mμ intervals from 700 to 800 mμ were practically linear. Thus, measurement of the rate of color change by reflectance at several temperatures would permit calculation of color stability using a method similar to that described by Garrett and Carper (11).

[1] Resistor normally supplied for use with Beckman flame photometer attachment.

[2] The portion of light to be measured that actually enters the phototube aperture.

[3] A grease-free, bentonite cream, containing sulfur and resorcinol as the primary therapeutic agents, prepared by the Purdue University School of Pharmacy Manufacturing Laboratory.

An experiment was designed to measure both the change in reflectance of Resorsul cream on aging at elevated temperatures and the effect of various concentrations of sulfur and resorcinol. The concentrations of sulfur and resorcinol were varied in an attempt to ascertain the cause of the color instability. A series of samples containing four different concentrations of both sulfur and resorcinol as shown in Table I were prepared.

TABLE I.—AMOUNTS OF SULFUR AND RESORCINOL USED IN PREPARING RESORSUL CREAM FOR STUDYING COLOR STABILITY

Cream No.	Sulfur	Resorcinol	Cream No.	Sulfur	Resorcinol
1	N[a]	N	5	N	2N
2	2N	N	6	N	N/2
3	N/2	N	7	N	0
4	0	N			

[a] N stands for the normal amount specified in the Purdue formula. Only the amounts of sulfur and resorcinol were varied.

The sample containers were one-half ounce green ointment jars.[4] Each jar was filled carefully to exclude air bubbles and covered with a two-inch square of Saran Wrap[5] before the cap was affixed. Samples of each cream listed in Table I were aged for various time intervals at room temperature and 55°, 60°, 65°, 70°, and 75°. These temperatures were obtained by the use of constant temperature ovens. All temperatures were observed to vary about ±0.5° except the 70° temperature which varied slightly less than ±1.0°. At selected time intervals, 10 samples—four of cream 1 and one each of the other creams—were removed from each oven

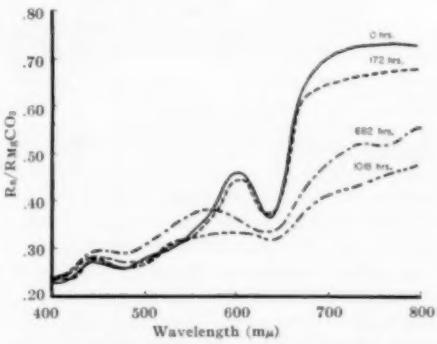

Fig. 1—Reflectance spectrum of Resorsul cream aged at 65° for various time intervals. R_s/R_{MgCO_3} is the ratio of the reflectance of the sample to that of $MgCO_3$. $R_{MgCO_3} = 1$. The times on the curves refer to aging time.

[4] Available from Owens-Illinois Glass Company, Toledo, O.

[5] A thin plastic covering made by Dow Chemical Co., Midland, Mich.

and placed in a refrigerator at 7° for about one and one-half hours in order to quench the reaction. The samples were then removed and allowed to equilibrate at room temperature.

Reflectance measurements on each sample were made at 700, 740, and 780 mμ. These wavelengths were chosen because this was the region where the logarithm of the rate of decrease in reflectance was a reasonably linear function of time as shown by preliminary experimentation. It was also the region in which the maximum changes in reflectance for a given time interval occurred (see Fig. 1).

Preliminary examination of the results revealed that an exponential curve was obtained when the reflectance values at 740 mμ for cream 1 were plotted against aging time. By plotting this change in reflectance against time on semi-logarithmic paper, straight lines were obtained as shown in Fig. 2. Each point is the mean value of four average reflectance readings, derived from three readings on each of four samples of cream 1. Although the lines drawn for each temperature seem to have the same *y*-intercept for a given concentration, the initial reflectance, R_o, was not considered in fitting the line to the data. Errors in starting the reaction at a given temperature at time zero make these points less accurate than the others (16, 17).

Data for the other creams were plotted in a similar manner and the slope of each line was determined. Table II shows the slopes for different creams at each temperature. The points for the samples of creams 2, 3, and 6 stored at 75°, and for all of cream 5 were so widely scattered that representative lines could not be drawn. The data in the table show that the slope is independent of the initial concentration of sulfur and resorcinol.

TABLE II.—SLOPES OF SEMI-LOGARITHMIC REFLECTANCE-TIME PLOTS FOR RESORSUL CREAM AT EACH AGING TEMPERATURE

Temp.[a]	Slope of Semi-log Reflectance-Time Plots[b]			
	Cream 1[c]	Cream 2[c]	Cream 3[c]	Cream 6[c]
75	0.0019	...	...	...
70	0.00070	0.00080	0.00076	0.00052
65	0.00042	0.00044	0.00043	0.00050
60	0.00020	0.00020	0.00020	0.00017
55	0.00006	0.00005	0.00009	0.00005

[a] Room temperature omitted, slope was approximately zero.

[b] Slopes were calculated by applying the standard two-point form of a straight line. Slopes for creams 2, 3, and 6 at 75°, and cream 5 at all temperatures could not be obtained (see text). Slopes for creams 4 and 7 were approximately zero, indicating that there was practically no change in reflectance when either resorcinol or sulfur was omitted. It is possible that some interaction between sulfur and resorcinol caused the color change.

[c] See Table I for description.

Rate constants for the decrease in reflectance of cream 1 were calculated from the slopes of the lines shown in Fig. 2. The general equation for the lines shown here was found to be

$$\log R_t = -kt/2.3 + \log R_o, \text{ where} \quad \text{(Eq. 1)}$$

R_t = the reflectance of the sample at time t, k = the rate constant, and R_o = the *y*-intercept. The slope of the line = $-k/2.3$.

Figure 3 shows that the graph of log k *vs.* $1/T$ is a straight line which is in accord with the Arrhenius theory (16), which relates rate constant to absolute

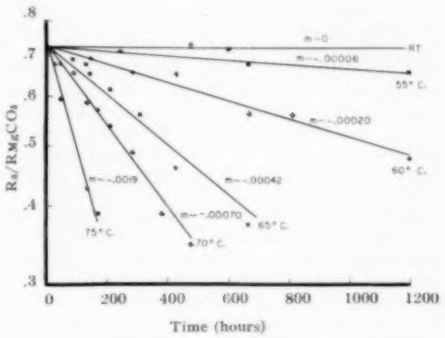

Fig. 2—Variation of R_s/R_{MgCO_3} at 740 mμ of normal Resorsul cream as a function of time and temperature. R_s/R_{MgCO_3} is the reflectance of the cream relative to that of $MgCO_3$. $R_{MgCO_3} = 1$ m is the slope of the line.

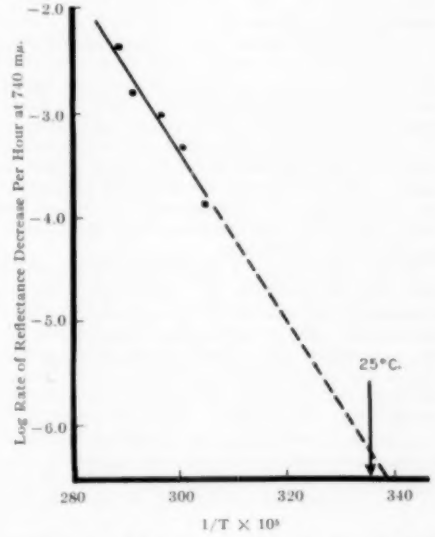

Fig. 3—Plot of the logarithm of the rate of decrease in reflectance per hour against the reciprocal of the absolute temperature (T) from data obtained at 740 mμ for the normal Resorsul cream samples.

temperature. The equation expressing this relationship is

$$\log k = -E/2.3\,RT + \log A, \text{ where} \quad \text{(Eq. 2)}$$

k is the rate constant; E, the energy of activation; R, the gas constant; T, the absolute temperature; and A, the frequency factor. By extrapolation, the rate constant (k) for the rate of decrease in reflectance of Resorsul cream with time at 25° was found to be 6.2×10^{-7} hours^{-1}.

Observations on the behavior of Resorsul cream at elevated temperatures have shown that when the reflectance, R_t, reaches 0.65, the cream no longer has a pleasing appearance. The predicted time for this estimated point of rejection may be found from Eq. 1. When the values 6.2×10^{-1} hrs.$^{-1}$, 0.65, and 0.722 were substituted into the equation for k, R_t and R_o, respectively, t was found to be approximately nineteen years. The value R_o was obtained from Fig. 2. It is obvious that the color of this preparation is quite stable. The purpose of this portion of the work was to illustrate the use of reflectance methods in determining color stability and not to evaluate this particular preparation.

Uniformity of Color in Coated Tablets.—The purpose of this portion of the project was to study a possible technique for measuring the reflectance of coated tablets. Such a method could be of considerable value in obtaining exact color matches from one batch of coated tablets to another. The main problem was to devise a method of reproducing the optical geometry for each reading.

The method developed consisted of drilling a hole in a $MgCO_3$ block the same diameter[6] as the tablet to be investigated and to a depth of approximately one-half the thickness of the tablet. After positioning the block so that the hole was exactly in the center of the aperture, both the block and the sphere were marked so that the position could be reproduced. By measuring the reflectance of a series of yellow coated tablets,[7] it was found that the error in repositioning the sample holder between each of 15 readings caused a standard deviation in reflectance of 0.00053. Slight irregularities in tablet shape produced standard deviations in reflectance of 0.00090, 0.00085, and 0.00074 when the reflectance of three different tablets was determined 15 times. The tablets were removed and replaced in the holder between each reading. Therefore, the errors caused by these two factors may be neglected for relative measurements.

To illustrate the use of reflectometry in measuring differences in reflectance of coated tablets, the reflectances of 30 tablets from two different lots[8] were determined. The average reflectances of the two lots were 0.8886 and 0.8747. Standard deviations were 0.0054 and 0.0058, respectively. Even though the difference of 1% in reflectance between the two batches was not visually detectable, the above methods did show that small differences in the reflectance of coated tablets could be measured.

Comparison of Color in Powders and Granules.—An investigation was undertaken to determine the applicability of reflectometric methods to the measurement of color in powders and granulations. A sample of a single shade of face powder manufactured by each of three different companies[9] was obtained. Eight Gm. of each powder sample were pressed into $^1/_4$-in. thick by $1^1/_8$-in. diameter pellets using a Carver hydraulic press. Reflectance measurements were made by the previously described methods using $MgCO_3$ as the reference with the sample pellet at the bottom aperture. Figure 4 shows the variation in reflectance that was obtained for the three samples. Visual observation of the

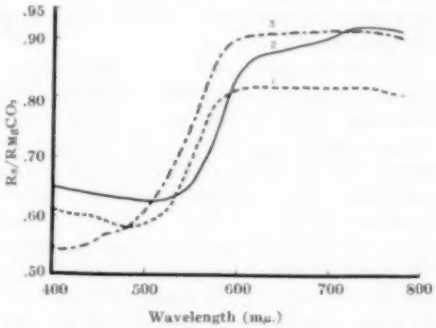

Fig. 4—Reflectance spectra of Rachel Face Powder. 1—Woodbury, 2—Lady Esther, 3—Ponds. R_s/R_{MgCO_3} is the reflectance of the sample relative to the reflectance of $MgCO_3$. $R_{MgCO_3} = 1$.

samples also indicated slight variation in color. Sample 1 was observed to be darker than the others; while sample 3, which had a slight yellow tint, was the lightest in color. Sample 2 appeared to be bluer than the others.

To demonstrate the possibility of measuring the reflectance of tablet granulations, the reflectance of a yellow sodium bicarbonate granulation both as granules and as a $^5/_{16}$-in. thick by $2^1/_4$-in. diameter pellet was determined. It was found that the reflectance spectrum for the pellet was 5 to 15% higher than that for the granules. This would seem to indicate that the reflectance of the granulation was somewhat dependent on the density of the sample.

The above examples clearly demonstrate the application of reflectometry to measuring light reflected from various types of pharmaceuticals. These techniques should prove applicable to problems of color control, matching and stability in opaque solid and semisolid pharmaceuticals.

SUMMARY AND CONCLUSIONS

1. An integrating sphere reflectance attachment for the Beckman DU spectrophotometer has been designed for the purpose of studying applications of reflectometry to pharmaceutical research.

2. The apparatus has been used to measure

[6] Less than $^1/_{64}$ inch clearance.
[7] Mi-Cebrin (Lilly), Lot. No. 5139-671106. One-half inch in diameter by $^9/_{32}$ in. thick.
[8] "Mi-Cebrin" (Lilly), Lot Nos. 5139-671106 and 2136-663079 NU.
[9] "Rachel" face powder made by Lady Ester, Ponds, and Woodbury.

the decrease in reflectance with time of a cream aged at room temperature and 55°, 60°, 65°, 70°, and 75° as an example of its use in determining color stability of opaque solids and semisolid pharmaceuticals.

3. Rate constants for this decrease in reflectance were obtained from the slopes of lines plotted for each temperature. Using the Arrhenius relation of rate constant to absolute temperature, the 25° rate constant was found by extrapolation of a plot of log k *vs.* $1/T$. This value of k has been used to predict the color stability of a representative cream.

4. Reflectance of coated tablets has been investigated. A method for accurately reproducing the optical geometry for each reading has been described. An example showing the use of these methods for the measurement of small differences in reflectance of coated tablets was given.

5. The possible application of reflectance measurements to color control in powders and granules has also been studied using face powders and a sodium bicarbonate tablet granulation as examples.

REFERENCES

(1) Hand, D. B., Robinson, W. B., Wishnetsky, T., and Ransford, J. R., *J. Agr. Food Chem.*, **1**, 1209(1953).
(2) Gillett, T. R., and Holven, A. L., *Ind. Eng. Chem.* **35**, 210(1943).
(3) Jacquez, J. A., and Kuppenheim, H. F., *J. Appl. Physiol.*, **7**, 523(1955).
(4) Jacquez, J. A., *et al.*, *ibid.*, **8**, 297(1955).
(5) Jacquez, J. A., *et al.*, *ibid.*, **8**, 212(1955).
(6) McCready, R. A., and McComb, E. A., *Anal. Chem.*, **26**, 1645(1954).
(7) Fischer, R. B., and Vrátný, F., *Anal. Chim. Acta*, **13**, 588(1955).
(8) McNicholas, H. J., *Bur. Standards J. Research*, **1**, 29 (1928).
(9) Stenius, A. S., *Svensk Papperstidn.*, **54**, 663(1951).
(10) Judd, D. B., "Color in Business, Science and Industry," 1952, pp. 314–18, John Wiley and Sons, Inc., New York.
(11) Garrett, E. R., and Carper, R. F., THIS JOURNAL, **44**, 515(1955).
(12) Jacquez, J. A., *et al.*, *J. Opt. Soc. Amer.*, **45**, 971(1955).
(13) Tellex, P. A., and Waldron, J. R., *ibid.*, **45**, 19(1955).
(14) National Bureau of Standards Letter Circular LC-547, 1939 National Bureau of Standards, Washington, D. C.
(15) Jacquez, J. A., and Kuppenheim, H. F., *J. Opt. Soc. Amer.*, **45**, 460(1955).
(16) Frost, A. A., and Pearson, R. G., "Kinetics and Mechanisms," John Wiley and Sons, Inc., New York, 1953, pp. 27–53.
(17) Livingston, R. (In S. L. Friess and A. Weissberger, "Investigation of Rates and Mechanisms of Reactions," Interscience Publishers, Inc., New York, 1953, p. 45.)

Aqueous Oral Preparations Containing Ascorbic Acid, Vitamin B_{12}, and Ferrous Gluconate*

By C. F. GERBER, C. P. HETZEL, O. KLIOZE, and A. F. LEYDEN

Ascorbic acid, vitamin B_{12}, and ferrous gluconate, although mutually incompatible, may be compounded into stable, aqueous oral preparations by using commercial 70 per cent sorbitol solution as a vehicle. Data are presented showing the stabilizing effects of sorbitol in two formulations containing ascorbic acid, vitamin B_{12}, and ferrous gluconate, and in a multivitamin formulation from which ferrous gluconate was excluded. Stability of these products is attributed specifically to their sorbitol vehicles.

ASCORBIC ACID, vitamin B_{12}, and ferrous iron salts are mutually incompatible. The incompatibility of vitamin B_{12} with ascorbic acid in aqueous solution is extreme. Complete loss of cyanocobalamin and ascorbic acid, for example, was observed in an aqueous solution stored for five weeks at 25°. Similar studies (1–3) of such solutions have emphasized the serious nature of the vitamin B_{12}-ascorbic acid incompatibility, although activity loss was not as marked.

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The incompatibility between ferrous iron salts and vitamin B_{12} is less pronounced. In our studies an aqueous solution (pH 4) of cyanocobalamin and ferrous gluconate lost 25 per cent of its vitamin B_{12} activity after five weeks' storage at 37°, 44 per cent after three months' storage and 96 per cent after nine months' storage at room temperature. Another aqueous solution of ferrous sulfate and cyanocobalamin showed 55 per cent loss of vitamin B_{12} activity after 5 weeks' storage at 37°.

While the destructive action of iron salts on ascorbic acid is generally known, little has been reported concerning the extent of this incompatibility. Ferric iron salts cause more destruction

than ferrous iron salts, but both are extremely deleterious to vitamin C in aqueous solution. A solution of ascorbic acid and ferrous gluconate, adjusted to pH 4.2 and stored for two weeks at 37°, lost 28 per cent of its activity, whereas a corresponding solution which contained an equivalent amount of iron in the form of ferric ammonium citrate lost 61 per cent activity. After four weeks' storage at 37° both solutions showed an 82 per cent loss of activity.

Investigations in our laboratories were directed toward preparing practical aqueous dosage forms containing vitamins B_{12} and C. The effect of ferrous gluconate on stability of the vitamins was also considered.

Initially, the relationship of low water content of such preparations to stability of the vitamins was studied. Multivitamin solutions were prepared using aqueous glycerin (67–70 per cent glycerin) as a vehicle. These formulations were not satisfactorily stable as will be discussed later. Furthermore, liquid formulations containing high concentrations of glycerin are quite unpalatable.

Attention then turned to 70 per cent sorbitol solution as a vehicle for preparations of this type. Commercial sorbitol solution (Sorbo®)[1] has a low water content and possesses a pleasant, sweet taste. It was found that use of 70 per cent sorbitol solution, either alone or with glycerin, as a vehicle for oral liquid products containing ascorbic acid, vitamin B_{12}, and ferrous gluconate circumvents the mutual incompatibilities of this combination.

Two formulations containing this combination and a multivitamin liquid, which does not include ferrous gluconate, are discussed here. The composition of these products is given in Table I.

EXPERIMENTAL

Formulation A (Hematinic).—The hematinic preparation was the simplest of the three preparations. It contained ferrous gluconate, vitamin B_{12}[2], and ascorbic acid; 70% sorbitol solution alone was used as the vehicle.

The ferrous gluconate was dissolved in the 70% sorbitol solution at 70°. The temperature was dropped to 45° and the ascorbic acid was dissolved. The solution was cooled to room temperature and the vitamin B_{12} was added. The solution was finally adjusted to pH 4.0 with sodium citrate and flavored.

Formulation B (Multivitamin Drops).—The multivitamin drops contained no iron salt because of the high concentration required for adequate iron dosage in 0.6 cc. and because of incompatibility with vitamin A. This dosage form contained vitamin B_{12} and ascorbic acid, with vitamins A and D and B-complex vitamins. The vehicle was composed of three volumes of 70% sorbitol solution and one volume of glycerin.

The niacinamide and riboflavin-5′-phosphate sodium were dissolved in 70% sorbitol solution at 45°. This temperature was maintained and the ascorbic acid was dissolved. The rest of the B-vitamins, including vitamin B_{12}, were dissolved in this solution at room temperature.

Vitamin A Palmitate for Aqueous Dispersion (Vapad®, Pfizer) and vitamin D_2 in corn oil were mixed and diluted further with a small amount of corn oil. This oil solution was then used to form a primary emulsion according to the National Formulary X method for cod liver oil emulsion. One part of powdered acacia (by weight) was dispersed in four parts of oil solution containing vitamins A and D. Two parts of 70% sorbitol solution (by weight) were added quickly and emulsification was accomplished using a Waring Blendor.

TABLE I

	Concn./Dose	Concn./cc.
Formulation A (Hematinic); Dose = 5 cc.		
Vitamin B_{12}, mcg.	2	0.4
Ascorbic acid, mg.	100	20
Ferrous gluconate, mg.	85	17
Vehicle: Sorbo;[a] flavors; pH, 4.0		
Formulation B (Multivitamin Drops) Dose = 0.6 cc.		
Vitamin B_{12}, mcg.	1	1.67
Ascorbic acid, mg.	50	83.3
Vitamin A, units	5,000	8,333
Vitamin D, units	1,000	1,667
Thiamine hydrochloride, mg.	1	1.67
Riboflavin,[b] mg.	1	1.67
Pyridoxine hydrochloride, mg.	1	1.67
Niacinamide, mg.	10	16.7
Panthenol, mg.	2	3.33
Vehicle: Sorbo, 75% (v/v); glycerin, 25% (v/v); acacia, 4 mg./cc.; corn oil, q.s.; flavors; pH, 4.0–4.5		
Formulation C (Tonic); Dose = 10 cc.		
Vitamin B_{12}, mcg.	5	0.5
Ascorbic acid, mg.	100	10
Ferrous gluconate, mg.	60	6
Liver fraction 1, mg.	50	5
Folic acid, mg.	0.33	0.033
d-Amphetamine sulfate, mg.	1	0.1
Methyl testosterone, mg.	2	0.2
Ethinyl estradiol, mcg.	2	0.2
Vehicle: Sorbo, 67.5% (v/v); glycerin-CMC,[c] 22.5% (v/v); ethanol, 10% (v/v); flavors; pH, 4.0–4.2		

[1] Registered trade-mark, Atlas Powder Company, Wilmington, Del.

[2] Vitamin B_{12} used in this study was Cyanocobalamin, U. S. P. XV.

[a] Registered trade-mark, Atlas Powder Company, Wilmington, Del.

[b] As riboflavin-5′-phosphate sodium (riboflavin-5′-phosphate ester monosodium salt dihydrate, Pfizer).

[c] Registered trade-mark, Hercules Powder Company, Wilmington, Del.; 2% solution of sodium carboxymethylcellulose 70 Premium-Low in glycerin.

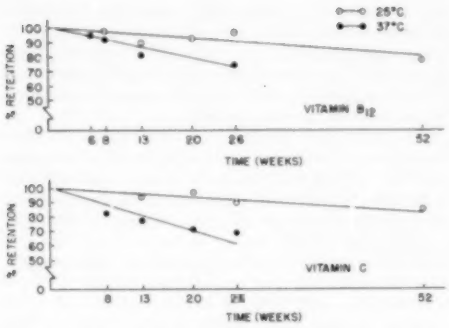


Fig. 1.—Formulation A—Stability of vitamins B_{12} and C at 25° and 37°.

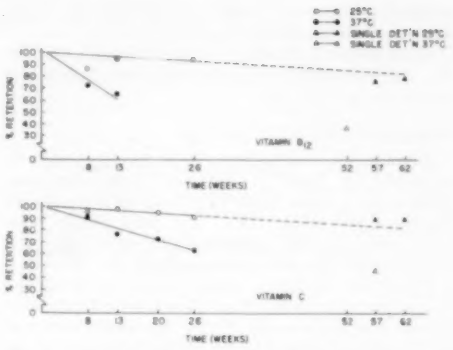


Fig. 2.—Formulation B—Stability of vitamins B_{12} and C at 25° and 37°.

The primary emulsion was then gradually diluted under gentle agitation with the 70% sorbitol solution containing the water-soluble vitamins. The solution was then adjusted to pH 4.0–4.5 with sodium citrate and flavored. The glycerin was added as the final step.

Formulation C (Tonic).—The tonic contained ferrous gluconate, vitamin B_{12}, and ascorbic acid, with other hematinic factors and additional components intended to impart a sense of well-being. The vehicle contained 10% ethanol; the remaining 90% of the vehicle was made up of three volumes of 70% sorbitol solution and one volume of glycerin, which contained 20 mg. sodium carboxymethylcellulose[3] per cc. glycerin.

The ferrous gluconate was dissolved in the 70% sorbitol solution at 70°. The temperature was reduced to 45° and the ascorbic acid was dissolved. After cooling the solution to room temperature, folic acid and thyroxine, dissolved in a minimum volume of 0.1 *N* sodium hydroxide, were added. The steroids, amphetamine sulfate, and vitamin B_{12} were dissolved in the ethanol and dispersed in the main solution. Sodium citrate was used to adjust the preparation to pH 4.0–4.2 and flavors were added.

Finally, the 2% solution of sodium carboxymethylcellulose in glycerin was added. The CMC® was included to restore the viscosity which was reduced sharply by the presence of ethanol.

Storage and Assay.—In all three formulations, principal interest was in the retention of potency by vitamins B_{12} and C which are reputedly unstable when present in the same dosage formulation. In the multivitamin drops (Formulation B) occasional assays were performed on other labile components, notably thiamine and vitamin A.

The samples were subdivided into one-ounce, screw-capped bottles with no further precautions. The bottles were stored in ovens at 37° and in an air-conditioned room maintained at 25°.

Generally, assays were performed after storage for 13, 26, and 52 weeks. Some lots were also assayed at 8 and 20 weeks.

Ascorbic acid was assayed according to the method in U. S. Pharmacopeia XV for Decavitamin Tablets with a peroxide modification. Cyanocobalamin was assayed microbiologically using a minor modification of the U. S. P. XV method.

[3] CMC-70 Premium Low, Hercules Powder Company, Wilmington, Del.

RESULTS

Stability at 25° and 37° of vitamin B_{12} and ascorbic acid in these formulations is shown in Figs 1–3. Each point on these graphs usually represents an average of assays from nine individual batches of the specified formulation. Straight lines drawn through these points by inspection indicate decomposition trends of the vitamins.

Stability at 25°

Formulation A (Hematinic).—Vitamin B_{12} lost 20% of its initial activity after one year's storage. During this period ascorbic acid lost 19% activity (Fig. 1).

Formulation B (Multivitamin Drops).—After six months' storage 8% losses of vitamin B_{12} and ascorbic acid were observed (Fig. 2). Only a single batch of this formulation was assayed after 57 and 62 weeks' storage. The proximity of these points to the extrapolated decomposition trend lines is noteworthy.

Vitamin A also showed an 8% loss after six months. It is interesting that thiamine hydrochloride proved to be the least stable vitamin in this

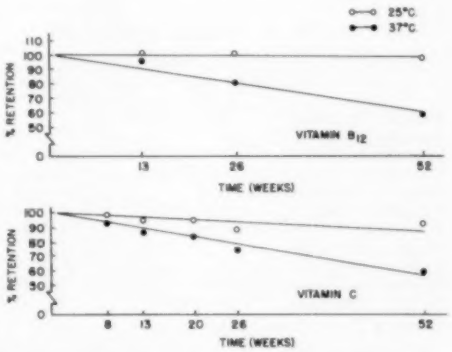


Fig. 3.—Formulation C—Stability of vitamins B_{12} and C at 25° and 37°.

product. There was an average loss of 50% activity after one year's storage

Formulation C (Tonic).—Losses of 3% for vitamin B_{12} and 14% for ascorbic acid were found after one year's storage (Fig. 3).

Stability at 37°.

Vitamin B_{12} and ascorbic acid decomposition in formulations stored at 37° was similar but progressed at a more rapid rate than in preparations stored at 25° (Figs. 1–3). The data at 37° were not correlated with stability behavior at 25°, and are presented solely for information.

Results of assays on single batches of Formulation B for vitamin B_{12} after 12 months' storage, and for ascorbic acid after 57 weeks' storage are shown in Fig. 2. These results indicate that the slopes of the estimated decomposition trend lines for this product at 37° are inordinately steep.

In our laboratories accelerated aging tests on vitamin preparations have been found useful as a means of determining gross incompatibilities in such formulations, and, when exceptional stability is exhibited at an elevated temperature, as an indication of "room temperature" stability. Tests of this type are also useful in estimating overages in finished dosage forms to compensate for variability of "room temperature" shelf storage conditions. However, an accurate measure of a product's shelf stability is obtained only on samples stored at 25° or at "room temperature."

DISCUSSION

The only component common to Formulations A, B, and C, other than ascorbic acid and vitamin B_{12}, is commercial 70% sorbitol solution. Stability of these formulations is attributed specifically to their sorbitol vehicles. Table II shows overages suggested for maintenance of labeled activity of these products for one year at room temperature, based on their behavior at 25°.

TABLE II.—SUGGESTED OVERAGES[a]

	Vitamin B_{12}		Ascorbic Acid	
	Activity Retention[b] %	Overage, %	Activity Retention[b] %	Overage, %
Formulation A	80	25	81	25
Formulation B	84[c]	20	84[c]	20
Formulation C	97	10	86	20

[a] Sufficient to maintain labeled activity for one year at room temperature.
[b] Activity retention as determined after storage for one year at 25°.
[c] Activity retention estimated for one year at 25°.

As mentioned earlier, glycerin-water systems proved unsuccessful as vehicles for liquids containing ascorbic acid and vitamin B_{12}. Two multivitamin formulations containing 33 and 30% (v/v) water (Formulations D and E) were studied in this connection (Table III).

After 6 months' storage at room temperature, two batches of D lost an average of only 11% of vitamin B_{12}, but 51% of ascorbic acid; after 12 months' storage, these losses had increased to 29% for vitamin B_{12} and 98% for ascorbic acid.

Opposite results were found with two batches of E. After storage for 3 months at room temperature, the average loss of ascorbic acid was only 3%, but there was a 78% loss in vitamin B_{12} activity.

The good stability performance of ascorbic acid and vitamin B_{12} in 70% sorbitol solution may be attributed to two possible causes: the reduced availability of water in sorbitol solution or complex formation between the polyol and either or both of the vitamins.

The specific gravity of Sorbo is about 1.3; thus, 100 cc. contains 39 Gm. water. Since vitamins B_{12} and C showed good stability in Formulations A, B, and C and not in the glycerin preparations (D and E), it follows that the water in the former products may not be as available to cause chemical interaction between the vitamins.

Notably, Formulation A, in which 70% sorbitol solution alone constituted the menstruum, contained more water per unit volume than Formulations D or E (39 cc./100 cc. vs. 33 and 30 cc./100 cc.). Thus, it cannot be concluded that the absolute water content of Formulations A, B, and C fully explains the stabilizing effect of sorbitol.

Reduction of reactive water through water-binding by sorbitol could be involved in stability of these formulations. Sorbitol has humectant properties. Solutions of sorbitol lose water more slowly than do glycerin solutions when transferred from high to low humidity conditions (4). This indicates that sorbitol may "bind" water more tenaciously than glycerin to make it less available for

TABLE III

	Formulation D; Dose = 5 cc. Concn./Dose	Concn./cc.	Formulation E; Dose = 0.6 cc. Concn./Dose	Concn./cc.
Vitamin B_{12}, mcg.	10	2	3	5
Ascorbic acid, mg.	40	8	30	50
Vitamin A, units	5,000	1,000	5,000	8,333
Vitamin D, units	1,000	200	1,000	1,667
Thiamine hydrochloride, mg.	2.0	0.4	2	3.33
Riboflavin, mg.	1.2	0.24	0.5[a]	0.83[a]
Pyridoxine hydrochloride, mg.	1.0	0.2	1.0	0.67
Niacinamide, mg.	20	4	20	33.3
Calcium pantothenate, mg.	3	0.6	3[b]	5[b]
Gentisic acid ethanolamide	1.2% (w/v)			
Sodium benzoate	0.2% (w/v)		0.2% (w/v)	
Vehicle[c]				

[a] As riboflavin-5'-phosphate sodium (riboflavin-5'-phosphate ester monosodium salt dihydrate, Pfizer).
[b] Panthenol was used instead of calcium pantothenate.
[c] Formulation D: water, 33% (v/v); Tween 80, 5% (v/v); glycerin, q.s.; pH 4.2; Formulation E.: water, 30% (v/v); Tween 80, 10% (v/v); glycerin, q.s.; pH 4.4.

chemical interaction between the vitamins. The water in starch, for example, tends less to induce or foster a given chemical action than an equivalent amount of free distilled water. A similar phenomenon may be part of the stabilizing effect observed when 70% sorbitol solution is used as a vehicle for ascorbic acid, vitamin B_{12}, and ferrous gluconate, and would explain the apparent anomaly which exists between Formulations A, B, and C and the glycerin preparations, D and E, when only the water content of these products is considered.

The other possible explanation for stability of these formulations may lie in complex formation between sorbitol and either or both ascorbic acid and vitamin B_{12}. It is also conceivable that water-binding and complex formation by sorbitol contribute jointly toward stability of these products.

REFERENCES

(1) Gakenheimer, W. C., and Feller, B. A., THIS JOURNAL, **38**, 660(1949).
(2) Bartilucci, A., and Foss, N. E., *ibid.*, **43**, 159(1954).
(3) Hutchins, H. H., Cravioto, P. J., and Macek, T. J., *ibid.*, **45**, 806(1956).
(4) "Guide to the Use of Atlas Sorbitol and Surfactants in Cosmetics," Atlas Powder Company, Chemicals Division, Wilmington 99, Del., copyright, 1956.

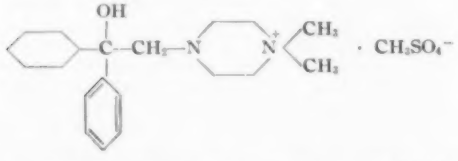
Methods for the Determination of Tral[1], a New Anticholinergic Drug*

By PRESTON F. HELGREN, JAMES G. THEIVAGT, and DONALD J. CAMPBELL

Tral, a new compound of the quaternary ammonium group of anticholinergic compounds, has the property of forming a colored chloroform soluble complex with bromthymol blue or ammonium cobaltothiocyanate. Under proper conditions, the amount of complex extracted is linearly related to the amount of Tral present: from 0.025 mg. to 0.350 mg. for the bromthymol reaction, and from 0.05 to 3.0 mg. for the ammonium cobaltothiocyanate reaction. Depending upon the nature of other substances present, either method provides an analytical method reproducible to ± 1.0 per cent.

RECENTLY A NEW ANTICHOLINERGIC DRUG of the quaternary ammonium group has become available.

The pharmacology of the drug has not been described in detail but it has been compared with other synthetic quaternary ammonium anticholinergic compounds by Hwang (1). Details of its synthesis will be reported later. The structure of the drug is as follows:



Physical Properties.—The compound is soluble in water, 50 per cent w/v, slightly soluble in chloroform and insoluble in ether. The melting point (Class I, U. S. P.) is between 200–210°, but characteristic of this group of compounds, it does not melt sharply. The compound has only slight absorption in the ultraviolet region, due to benzenoid absorption. In 0.1 *N* sulfuric acid there are well defined maxima at 252 mμ, 257mμ and 263 mμ, the absorptivity (1 per cent, 1 cm.) at 257 mμ being 4.

The infrared spectrum of a Nujol mull preparation of the compound was determined using a Perkin-Elmer double beam spectrophotometer. The resulting spectrum[2] is shown in Fig. 1.

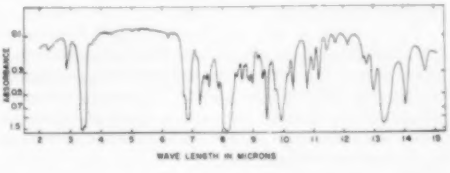


Figure 1.

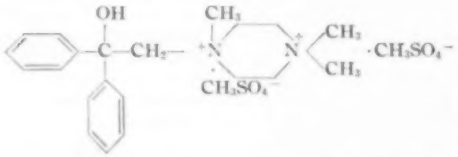
* Received April 8, 1957, from the Control Department Abbott Laboratories, North Chicago, Ill.
The authors wish to acknowledge the helpful suggestions of Dr. William B. Brownell, Analytical Research Group, Control Department.
[1] Tral is the trademark of Abbott Laboratories' hexocyclium.

[2] The infrared spectrum was determined by William H. Washburn, Control Department, Abbott Laboratories.

METHODS OF ANALYSIS

Due to various problems of interference that arose from tablet excipients, three different methods of analysis were studied and developed. Because of the low intensity of the ultraviolet spectrum, it is of use only as an identity for the drug. The methods studied are discussed below.

Non-Aqueous Titration.—When Tral is dissolved in glacial acetic acid, it can be titrated with 0.1 *N* perchloric acid in acetic acid. However, only one equivalent per mole is titrated. This titration involved the nonquaternized nitrogen in the piperazine ring of Tral, since the similar *completely* quaternized compound shown below does not titrate under these conditions.



As Tral is a salt of mono-methyl sulfate, the failure to titrate this group with perchloric acid indicates that the methyl sulfate anion is a weaker base than the acetate ion. In this respect, Tral is similar to the quaternary ammonium halides which cannot be titrated until the weakly basic chloride anion is replaced by acetate ion using mercuric acetate (2, 3).

As the perchloric acid titration involves only the non-quaternized nitrogen, this method of assay is a reliable means of determining any over-quaternization that may take place during manufacture, as well as a method of analysis for certain tablets.

Titration Procedure.—Weigh 20 tablets and grind. Transfer an amount of powder approximately equivalent to 250 mg. of Tral to a beaker containing 50 ml. of glacial acetic acid. Titrate with 0.1 *N* perchloric acid, using a potentiometric titrator with silver-glass electrodes. The break at the endpoint is about 40 millivolts per 0.1 ml. of titrant. The equivalent weight of Tral is 428.6.

This procedure was rapid and suitable for tablets not containing salts of weak acids which would also be titrated. However, certain tablets were prepared containing sodium citrate and magnesium stearate as excipients and as no organic solvent could be found to extract the Tral preferentially, the need arose for another method.

Photometric Determination by Compound Formation with Bromthymol Blue.—The use of indicators for the determination of quaternary ammonium salts was originally described by Auerbach (4). A review of photometric procedures has been made by Ballard (5). The procedures are all based upon the reaction of a dye containing a large negatively charged ion (anionic dye) with a quaternary compound, having a large cation, to form a complex soluble in an organic solvent. The technique we decided to investigate was the partition technique. According to Mukerjee (6) who has studied the theory and variables involved, there exists a series of equilibria that depend upon pH, the concentration of the dye, the organic solvent used, shaking time, and the ratio of the volumes of the two phases. All of these must be considered in the development of a sensitive and reproducible method. We first attempted to apply a method for quaternary ammonium compounds, which employs Eosin Y and a partitioning between tetrachloroethane and pH 4.5 buffer. This method was not applicable. Secondly, bromthymol blue was investigated and was found satisfactory, using chloroform as the extractant. Ballard (5) recommended that the amount of indicator extracted in chloroform be read at 420 mμ following the addition of a solution of boric acid in ethanol. In this solution the dye is in the yellow or acid form and has a molar absorptivity of 5.7×10^3 at 420 mμ. However, a chloroform solution of the dye made alkaline with 0.005 *N* sodium hydroxide in methanol is stable for several hours and the molar absorptivity is increased approximately ten-fold to 3.6×10^4 at 620 mμ. From this a 3.6×10^{-5} *M* solution of Tral would give an absorbance reading of 0.1 in a 1-cm. cell, assuming complete extraction and a 1:1 mole ratio of dye to Tral in the complex.

TABLE I.—EFFECT OF pH ON EXTRACTION OF TRAL-BROMTHYMOL COMPLEX

	Absorbance at 620 mμ of $CHCl_3$ Following Single Extraction								
pH of Buffer	6	6.5	7.0	7.5	8.0	8.5	9.0	10.0	12.0
Tral + Indicator	1.08	0.787	0.691	0.679	0.668	0.670	0.625	0.583	0.015
Indicator	0.80	0.158	0.027	0.012	0.002	0	0	0	0
Difference	0.28	0.629	0.664	0.667	0.666	0.670	0.625	0.583	0.015

In this case a quantitative estimation of as little as 1 ppm. of Tral should be possible, a sensitivity that could prove useful for work on biological samples.

The method applicable to the drug or tablets follows. *Reagents* and *Solutions:* bromthymol blue indicator solution—dissolve 150 mg. of bromthymol blue (acid form) and 150 mg. of sodium carbonate in water and dilute to 100 ml.; Tral standard solution—0.05 mg. per ml.; pH 8.5 buffer—dissolve 3.10 Gm. of boric acid and 3.73 Gm. of potassium chloride in water, adjust to pH 8.5 with sodium hydroxide, make up to 1,000 ml. with water; sodium hydroxide—0.005 *N* in methanol; chloroform—A.C.S. grade.

Take 20 tablets and grind to a fine powder. Transfer a sample equivalent to 100 mg. of Tral to a 100-ml. volumetric flask. Add 50 ml. of water, shake vigorously, then make up to volume. Allow the excipients to settle, or centrifuge an aliquot. Dilute 5 ml. of the clear supernatant to 100 ml. with water. Transfer a 5-ml. aliquot to a 125-ml. sepa-

ratory funnel having a Teflon stopcock. Add 1 ml. of bromthymol blue indicator solution and 15 ml. of pH 8.5 buffer solution. Add exactly 25 ml. of chloroform and shake on a mechanical shaker for ten minutes. After allowing the layers to separate, swirl the separatory funnel gently as the extracted complex in the chloroform layer tends to concentrate near the interface. Filter the chloroform layer through not more than 0.1 Gm. of glass wool into a flask. Transfer exactly 10 ml. of the chloroform filtrate into a test tube containing 2 ml. of 0.005 *N* sodium hydroxide in methanol and mix thoroughly. Determine the absorbance of this solution at 620 mμ in a Beckman DU Spectrophotometer using chloroform to set the instrument to 100% transmittance. Five milliliters (0.25 mg.) of the Tral standard solution are also run through the procedure starting with "Transfer a 5-ml. aliquot to a 125-ml. separatory funnel." Calculate the results by a comparison of the absorbances of the standard and a sample.

As pointed out by Mukerjee (6), to acquire an understanding of the variables concerned in the use of a partition method such as the above, a consideration of the various equilibria must be made. The following schematic representation of the system being studied (Fig. 2), also devised by Mukerjee is useful.

Bromthymol blue, the anionic dye, is DNa. Tral, the cationic compound, is RX. Thus R^+ is the quaternary cation and D^- is the colored dye ion. When mixed together in the two phase chloroform-water system the equilibria involved are as follows:

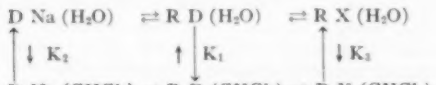


Fig. 2.—Schematic Diagram of Equilibrium.

The equilibria K_2 and K_3 represent the partitioning of dye and Tral between water and $CHCl_3$. The choice of solvent, the pK of the indicator and the pH of the aqueous phase are of prime importance in favoring the partition into $CHCl_3$, which in turn will increase the sensitivity of the analysis. The partitioning of the Tral-dye complex depends upon equilibrium K_1, and for optimum sensitivity conditions should be chosen to favor the chloroform phase. Certain of these variables were studied.

Effect of pH.—A series of separators was set up, each containing 0.25 mg. of Tral (0.58 micromoles) and 1.5 mg. of bromthymol blue (2.4 micromoles). To each separator was added 15 ml. of a different buffer, ranging from pH 6.0 to 12.0. The samples were shaken for a fixed period of time with chloroform and the absorbance of the indicator in the chloroform layer was determined as previously described. A similar series was run with indicator and buffer, omitting Tral.

The values obtained for extraction of indicator alone are a reflection of equilibrium K_2 as shown in Fig. 2 and the pK of the indicator which is 7.0. Based on this alone, a pH of 8.5 or higher would be desirable to eliminate a blank correction. The absorbance readings in the difference column show the amount of Tral-bromthymol complex extracted. This is directly related to equilibrium K_1 which reflects the degree of partitioning of the complex as well as the completeness of the Tral-dye complex formation. These optimum conditions prevail between pH 7.0 and 8.5.

From a consideration of these data it was decided to extract at pH 8.5. At this pH, the indicator blank is zero which eliminates the necessity of running a blank.

Shaking Time.—Next a study was made of the possible effect of shaking time on the reproducibility of single extraction at pH 8.5. Four samples of Tral were extracted at each shaking time. The absorbances obtained are shown in Table II.

TABLE II.—EFFECT OF SHAKING TIME UPON EXTRACTION OF TRAL-BROMTHYMOL COMPLEX

5 Minutes	Range	10 Minutes	Range	15 Minutes	Range	30 Minutes	Range
0.649		0.668		0.667		0.643	
0.663	0.092	0.674	0.006	0.659	0.016	0.645	0.010
0.741		0.669		0.675		0.640	
0.701		0.672		0.662		0.650	

For $n = 4$, the range of absorbances is not significantly different between ten minutes and thirty minutes shaking, so ten minutes shaking time was adopted as standard procedure.

Interfering Materials.—Common tablet excipients such as starch, magnesium stearate, polyvinylpyrrolidone, and sodium citrate were checked for possible formation of a chloroform soluble complex with bromthymol blue, but under the conditions of the procedure, no interference was found. However, certain tablets containing Carbowax® 6000[3] were formulated and it was found that at pH 8.5, 2 mg. of Carbowax combined with bromthymol blue to form a chloroform soluble complex giving an absorbance reading of 0.026 at 620 mμ. Extractions at lower pH gave variable but definitely higher readings. Thus the method is not suitable for a tablet containing Carbowax 6000.

Tral tablets containing 15 mg. of phenobarbital, U. S. P., have recently been analyzed by the above procedure and no separation of the barbiturate is necessary.

Relationship between Concentration of Tral and Absorbance Readings.—Samples containing between 0.01 mg. and 0.4 mg. of Tral per 5 ml. were extracted once with chloroform out of pH 8.5 buffer containing bromthymol blue as previously described, and the resulting absorbance at 620 mμ was measured. The results obtained and a statistical examination for linearity are shown in Table III.

[3] Trademark of Carbide & Carbon Co., polyethylene glycol.

TABLE III.—RELATIONSHIP BETWEEN CONCENTRATION OF TRAL AND ABSORBANCE READINGS

Actual mg. of Tral x	Absorbance of $CHCl_3$ at 620 mμ y	Absorbance Readings Calculated from Regression Line [a] $\hat{y}$
0.010	0.022	0.010
0.025	0.056	0.051
0.050	0.117	0.119
0.100	0.239	0.256
0.150	0.389	0.391
0.200	0.529	0.527
0.250	0.666	0.663
0.300	0.799	0.800
0.350	0.939	0.938
0.400	1.082	1.074

[a] $\hat{y} = a + bx$ $a = -0.017$ $b = 2.732$ $b = (n\Sigma xy - \Sigma x \times \Sigma y)/(n\Sigma x^2 - (\Sigma x)^2)$.

Comparison of the absorbance readings $\hat{y}$, calculated from the linear regression line, and y, the actual readings obtained, show from their small difference that Beers-Lambert law is followed for samples of Tral between 0.025 and 0.350 mg.

Reproducibility of Method.—Ten samples of 0.25 mg. of Tral were analyzed by the above procedure to determine the reproducibility of the method. $\bar{X}$, the average absorbance for $n = 10$ was 0.670, $R = 0.018$ and $\delta = \pm 0.006$. Thus the standard deviation of a single determination is about ±0.9%.

Composition of Tral-Bromthymol Complex.—As pointed out by Ballard (5), dye-quaternary compound complexes are formed in ratios other than 1:1. In our hands, attempts to determine this ratio by titration with Tral of a given amount of bromthymol in a pH 8.5 buffer-chloroform system until no color was left in the water layer, were not successful. This was probably due to the fact that for complete reaction, an excess of bromthymol blue is required.

Instead, varying amounts of Tral in pH 8.5 buffer were extracted once with chloroform in the presence of a fixed but always excess amount of bromthymol. The amount of dye left in the aqueous phase was determined by measuring the absorbance. The results obtained are given in Tablet IV.

TABLE IV.—MOLE RATIO OF DYE TO TRAL IN COMPLEX

Mole Ratio of Dye/Tral Present	Moles of Tral Added × 10^{-5}	Moles of Dye Removed × 10^{-5}
7.32	2.333	2.174
3.66	4.666	4.706
1.46	11.665	11.723

From these results it can be seen that although the excess amount of dye available for reaction varied from 1.5 to 7 times the amount of Tral present, the complex maintained a constant 1:1 dye/Tral composition. Previous studies had shown that a single chloroform extraction at pH 8.5 essentially removed all the Tral from the water phase as second and third extraction did not yield any more dye-Tral complex. The data in Table IV also support this.

Photometric Determination of Tral with Ammonium Cobaltothiocyanate.—As mentioned previously, it was found that when a pH 8.5 buffer solution containing Carbowax 6000 was shaken with bromthymol blue and chloroform, bromthymol blue or a complex of the dye and Carbowax is extracted into the chloroform layer. This of course would give high results for Tral in tablets containing Carbowax 6000 as an excipient. Brown and Hayes (7) described a method for the determination of polyethyleneglycol mono-oleate by the formation of a blue chloroform soluble complex of the polyglycol and ammonium cobaltothiocyanate. In chloroform, the concentration of the resulting complex can be determined by measuring the absorbance at 318.5 or 620 mμ. They also qualitatively studied this reaction using the Carbowax series from 200 to 4000 and noted that no chloroform soluble complex was formed above 1500. They also noted qualitatively that some quaternary ammonium compounds containing a long chain alkyl group gave a positive reaction. Thus it occurred to us that the method might be applicable to Tral in the presence of Carbowax 6000.

It was found that when a solution of Tral and ammonium cobaltothiocyanate was shaken in the presence of chloroform, a blue color appeared in the chloroform which has a peak at 620 mμ as reported by Brown and Hayes (7). We also confirmed their report that Carbowax 6000 did not impart a blue color to the chloroform layer. Thus the following procedure based on their method for polyethyleneglycol mono-oleate was found suitable for Tral and Tral-phenobarbital tablets.

Reagents.—Ammonium cobaltothiocyanate solution: dissolve 15 Gm. of Fisher certified reagent cobaltous nitrate and 100 Gm. of ammonium thiocyanate, A.R. in 500 ml. of H_2O.

Procedure.—To 20 ml. of the above reagent add 5 ml. of a solution of Tral adjusted to contain 1.5 mg. of the drug. Shake for one minute and let stand for five minutes. Add 5 ml. of chloroform and shake vigorously for one minute. Allow layers to separate. Pass the chloroform phase through a small pledget of glass wool. Determine the absorbance of the chloroform solution at 620 mμ in a suitable spectrophotometer. Calculate the results from a comparison of the absorbance of a known amount of Tral put through the same procedure.

DISCUSSION

Brown and Hayes (7) pointed out that the cobaltothiocyanate-polyglycol complex also exhibits a maximum in chloroform at 318.5 mμ which is six times as great as the maximum at 620 mμ. Contrary to their experience, we found that a water blank carried through their procedure gave an appreciable absorbance of 0.4 at 318.5 mμ. We confirmed that no blank correction was necessary at 620 mμ. The substance extracted has a peak at 263 mμ and thus it is probably not the $Co(CNS)_4^=$ anion whose measurement forms the basis of their method. We did note that our cobaltothiocyanate reagent had a pH of 6 while Brown and Hayes reagent was reported to be 3. Attempts to eliminate

this blank were not successful and at present this discrepancy must be attributed to differences in quality of the reagents.

Relationship between Concentration of Tral and Absorbance.—A series of Tral samples from 0.05 mg. to 3.00 mg. were reacted with the cobaltothiocyanate reagent and extracted once with chloroform. The resulting absorbances at 620 mμ were read and an examination of the results for statistical linearity is given in Table V.

TABLE V.—RELATIONSHIP BETWEEN CONCENTRATION OF TRAL AND ABSORBANCE READINGS

Actual mg. of Tral	Absorbance of $CHCl_3$ at 620 mμ	Absorbance Readings Calculated from Regression Line [a]
x	y	$\hat{y}$
0.05	0.016	.011
0.25	0.088	0.085
0.50	0.170	.178
1.00	0.355	.363
1.50	0.550	.548
2.00	0.730	.733
2.50	0.918	.918
3.00	1.095	1.103

[a] $\hat{y} = a + bx$, $a = 0.007$, $b = 0.0368$.

The above data show that the Tral-cobaltothiocyanate complex follows Beers-Lambert law between 0.05 and 3.0 mg. using only a single extraction. When second and third extractions were made it was found that the single extraction removes approximately 88% of the complex.

Reproducibility of Method.—Ten samples of 1.5 mg. of Tral were analyzed by the above procedure to determine the reproducibility of the method. $\bar{X}$, the average absorbance for $n = 10$ was 0.548, $R = 0.018$ and $\delta = \pm 0.006$. Thus the standard deviation for a single determination is about $\pm 1.0\%$.

Interfering Materials.—Common tablet excipients put through the procedure essentially did not produce any color in the chloroform. The results on these and a few other compounds are given in Table VI.

TABLE VI.—ABSORBANCE $CHCl_3$ AT 620 mμ

Compound	Amount, mg.	Absorbance
Magnesium stearate	1.0	0.000
Carbowax 6000	1.5	0.005
Pentobarbital Na	5.0	0.001
Methamphetamine HCl	5.0	0.002
Phenobarbital Na	5.0	0.000
Polyvinylpyrrolidone	2.0	0.003
Choline	2.0	0.004
Betaine	2.0	0.001

SUMMARY

1. Three methods have been described for the analysis of Tral, a new anticholinergic drug. They may be applied to Tral, Tral tablets, or Tral-phenobarbital tablets, depending upon the tablet formula.

2. The procedures involving the combination of Tral, with either bromthymol blue or ammonium cobaltothiocyanate to form a chloroform soluble complex, require specific reaction conditions to assure maximum sensitivity and good reproducibility.

REFERENCES

(1) Hwang, K., *Am. J. Gastroenterol.*, **26**, No. 1, 56(1956).
(2) Siggia, S., "Quantitative Organic Analysis Via Functional Groups," 2nd ed., John Wiley & Sons, New York, 1954, p. 118.
(3) Pifer, C. W., and Wollish, E. G., *Anal. Chem.*, **24**, 300(1952).
(4) Auerbach, M. E., *Ind. Eng. Chem., Anal. Ed.*, **15**, 492(1943).
(5) Ballard, C. W., Isaacs, J., and Scott, P. G. W., *J. Pharm. and Pharmacol.*, **6**, 971(1954).
(6) Mukerjee, P., *Anal. Chem.*, **28**, 870(1956).
(7) Brown, E. G., and Hayes, T. J., *Analyst*, **80**, 755(1955).

The Determination of Theophylline, Pentobarbital, and Papaverine in Tablets by Spectrophotometric Analysis*

By PRESTON F. HELGREN, FRANK E. CHADDE, and DONALD J. CAMPBELL

Tablets containing theophylline, pentobarbital, and papaverine may be analyzed rapidly and accurately by ultraviolet spectrophotometry. Following only the separation of pentobarbital, all three components may be determined accurately under conditions which studies have shown provide optimum reproducibility of absorbance measurements.

A CONTINUING TREND in the pharmaceutical industry is the preparation of dosage forms containing drugs of differing pharmacological action. This poses a problem to the analytical chemist because many of the methods in the official compendia apply only to dosage forms containing one active ingredient. Also for control purposes, it is desirable to perform the analyses by one type of procedure on the same sample, i.e., titrimetric, absorptimetric, or gravimetric.

This paper describes a method of analysis for a tablet containing Glucophyllin,[1] Nembutal,[2] and papaverine hydrochloride as the active ingredients. The procedure meets the above listed requirements in that all three components are determined on one sample by a common procedure, i.e., ultraviolet spectrophotometry.

EXPERIMENTAL

Reagents.—One per cent hydrochloric acid; 1.5% ammonium hydroxide; diethyl ether; pH 6.2 buffer prepared by mixing 80 ml. of $^2/_{15}$ molar KH_2PO_4 with 20 ml. of $^2/_{15}$ molar Na_2HPO_4 (double strength Sorensen buffer).

Procedure.—A typical formulation of this item contains 76 mg. of theophylline, 15 mg. of pentobarbital sodium, and 40 mg. of papaverine hydrochloride per tablet. Determine the average weight of 20 tablets and grind to a fine powder. Transfer the equivalent of one tablet to a 250-ml. separator containing 100 ml. of 1% hydrochloric acid. Shake for two minutes and extract with five 20-ml. portions of diethyl ether, collecting the ether extracts in another separator. Wash the combined ether extracts with three 10-ml. portions of slightly acid water. Add these water washes to the 1% hydrochloric acid remaining in the first separator. Save this solution for the analysis of theophylline and papaverine hydrochloride.

Determination of Pentobarbital Content.—Transfer the ether solution quantitatively to a 200-ml. volumetric flask and evaporate the ether without heat under a stream of nitrogen. Dissolve the residue in 1.5% ammonium hydroxide, shaking vigorously to assure complete solution. Dilute to volume with additional 1.5% ammonium hydroxide. If necessary, filter the solution through a pledget of glass wool. Transfer 10 ml. of the clear filtrate to a 50-ml. volumetric flask and dilute to volume with 1.5% ammonium hydroxide. Determine the absorbance in 1-cm. silica cells at 240 mμ in a Beckman DU Spectrophotometer against a blank of 1.5% ammonium hydroxide. Calculate the concentration of pentobarbital sodium from the following equation.

Mg. of pentobarbital sodium per tablet = (A240 mμ × 10,000 × Av. Wt. per Tablet)/(398 × Wt. of sample).

Determination of Theophylline and Papaverine.—Transfer the 1% hydrochloric acid solution quantitatively to a 200-ml. volumetric flask. Warm on the steam bath and blow gently with a stream of nitrogen to remove traces of diethyl ether. After cooling, dilute to volume with water. Transfer a 2-ml. aliquot to a 100-ml. volumetric flask and dilute to volume with pH 6.2 buffer. Determine the absorbance in 1-cm. silica cells at 243.5 mμ and 272 mμ using the buffer solution as blank. Calculate the concentration of theophylline and papaverine hydrochloride as follows:

Mg. of papaverine hydrochloride per Tablet = [(78.33 × A243.5 mμ − 18.45 × A272 mμ) × Av. Wt. per Tablet]/(Wt. of Sample).

Mg. of theophylline per Tablet = [(198.5 × A272 mμ − 23.44 × A243.5 mμ) × Av. Wt. per Tablet]/Wt. of Sample.

DISCUSSION

Method Development and Separation Conditions.—The U. S. P. XV procedures for papaverine hydrochloride injection and pentobarbital sodium were not suitable for this tablet. The first procedure attempted was to suspend the tablet mixture in 0.1 N sodium hydroxide and extract with chloroform. Although all of the papaverine was extracted into chloroform, about 20% of theophylline was also present. Further, upon heating the alkali layer (to remove traces of chloroform) this solution containing pentobarbital and theophylline turned yellow. This method was rejected because of the failure to separate papaverine and theophylline as well as the instability of theophylline in the alkali.

Next, the above procedure was repeated using diethyl ether. In this case, pentobarbital and theophylline remained in the alkali, but because of the

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[1] Glucophyllin is Abbott Laboratories' trademark for the methyl glucamine salt of theophylline.

[2] Nembutal is Abbott Laboratories' trademark for pentobarbital sodium.

low solubility of papaverine in ether, a quantitative separation was not achieved.

As both papaverine hydrochloride and theophylline are soluble in dilute acid, a separation of pentobarbital with ether was attempted. Because of the solubility of papaverine hydrochloride in chloroform, this solvent could not be used. Using synthetic blends of the three components, it was found that pentobarbital was extracted quantitatively into ether and both papaverine and theophylline remained in the dilute acid layer. When applied to tablets, a clean separation was also achieved with no emulsion problems.

Spectral Properties of Pentobarbital.—Aqueous solutions of pentobarbital, typical of 5,5 disubstituted barbiturates, have three distinct equilibrium forms between pH 1 and 14. Each form has a different ultraviolet spectrum, and dissociation constants of $pK_1 = 8.96$ and $pK_2 = 12.67$ have been calculated from them (1, 2). Fox and Shugar (3) have presented an excellent theoretical discussion of the equilibria involved and other authors (4–7) have utilized pH spectral changes to establish the identity of 5,5 disubstituted barbiturates in body tissues.

Thus, to determine pentobarbital by measurement of ultraviolet absorbance, the pH of the solution must be the same for the sample and the standard. Table I shows the shift in position of the pentobarbital maximum peak and change in absorptivity (1%, 1 cm.) in sodium hydroxide solutions of varying pH.

TABLE I.—SPECTRAL CHANGE OF PENTOBARBITAL WITH pH

Normality, NaOH	pH	Wavelength at Max. (mμ)	Absorptivity, (1%, 1 cm.)
0.0003	9.65	240	443
0.01	11.65	240	417
0.05	12.40	242	349
0.10	12.85	243	308
0.20	13.3[a]	247	290
0.50	13.7[a]	255	308
1.0	14.0[a]	256	323

[a] pH calculated.

All the above measurements were made in sodium hydroxide solutions adjusted with potassium chloride to a constant ionic strength (μ) of 1.0.

From the above, it is evident that for routine UV analysis sodium hydroxide solutions of pentobarbital are not suitable. Mattson (8) used pH 9.5 buffer for the UV determination of phenobarbital and McCallum (9) used ammonium hydroxide solution for the determination of the same drug in biological samples.

As buffer solutions are subject to mold growth and thus must be prepared frequently, it was decided to study the use of ammonium hydroxide solution of pentobarbital for UV analysis. Table II gives the results obtained.

TABLE II.—SPECTRAL PROPERTIES OF PENTOBARBITAL IN NH_4OH

Normality, NH_4OH	pH	Wavelength at Max. (mμ)	Absorptivity, (1%, 1 cm.)
0.10	11.10	240	436
0.50	11.52	240	434
0.75	11.52	240	437
1.0	11.65	240	437

From the above data it can be seen that within a large range of concentration of ammonium hydroxide, there is no shift in maximum and the absorptivity is constant. Also, pentobarbital solutions show good stability in ammonium hydroxide as the absorptivity decreased only 0.5% in twenty-four hours. Thus 0.75 *N* ammonium hydroxide (1.5%) was adopted as the solution to be used for the UV determination of pentobarbital in these tablets.

Spectral Properties of Theophylline and Papaverine.—In the procedure described, theophylline and papaverine are separated from pentobarbital in 1% hydrochloric acid. In this solution, the ultraviolet spectrum of papaverine has a single maximum at 251 mμ, absorptivity (1%, 1 cm.) of 1643, and theophylline a single maximum at 272 mμ, absorptivity (1%, 1 cm.) of 515. Also both substances exhibit minima of similar intensity near these two wavelengths. From this it was apparent that conditions were favorable for the UV determination of both substances without separation. Further, as the tablet contained approximately half as much papaverine as theophylline, the absorbance readings at the maxima would have approximately an 8:5 ratio, so that absorbance readings at both maxima would fall between the desired range of 0.3 and 0.7 on the same solution.

In the analysis of two component mixtures by UV there are certain conditions that will improve the accuracy of such analyses. Stearns (10) has pointed out that a mathematical examination of the familiar equations for determining concentration of mixtures of *A* and *B* simultaneously shows that the most accurate estimate of each is achieved when the ratios of the absorbances A/B and B/A at the maximum wavelength of each are maxima.

In 1% hydrochloric acid, theophylline has a narrow minimum at 243 mμ, absorptivity (1%, 1 cm.) of 123. At 251 mμ, the papaverine maximum, the theophylline curve is rising sharply and the absorptivity (1%, 1 cm.) on the side of the curve is 195. Thus, if it were possible for the papaverine maximum to be at 243 mμ, to coincide with the wavelength of the theophylline mimimum, the absorbancy ratio would be a maximum value. Papaverine, on the other hand, has a broad minimum in the region of the theophylline maximum (272 mμ) and optimum conditions for accuracy already prevail.

Foster and Macdonald (11) in a study of the ultraviolet spectrum of papaverine hydrochloride reported a shift of the maximum from 238 mμ at pH 9 to 251 mμ at pH 3.95. A change in absorptivity (1%, 1 cm.) was also observed. Thus based on Foster's data it is possible to shift the position of the papaverine maximum to 243 mμ so as to coincide with the position of the theophylline minimum. As pointed out earlier, an increase in precision would be achieved. Table III, page 646, gives the results of such a study.

Thus to achieve optimum precision in this two-component determination, the absorbance readings for papaverine and theophylline are made in pH 6.2 buffer. This increase in precision outweighed the

inconvenience of using the buffer. As the additive absorbances of mixtures of the two drugs followed the Beer-Lambert law, this procedure was adopted for the tablets.

TABLE III.—EFFECT OF pH ON UV ABSORPTION OF THEOPHYLLINE AND PAPAVERINE

pH	Max. Wavelength (mμ) Papaverine	Min. Wavelength (mμ) Theophylline
6.82	238	243
6.20	243.5	243
6.08	245	242
2.48	251	242

Reproducibility of Method.—A large number of tablets were powdered and ten separate samples were put through the described procedure. The results obtained are shown in Table IV.

TABLE IV.—STATISTICAL STUDY OF METHOD

	Pentobarbital Na	Theophylline	Papaverine Hydrochloride
X	14.7 mg.	74.9 mg.	39.7 mg.
n	10	10	10
R	0.4 mg.	1.9 mg.	1.0 mg.
δ	±0.11 mg.	±0.49 mg.	±0.26 mg.
$\delta\%$	±0.75%	±0.65%	±0.65%

SUMMARY

A spectrophotometric method for the determination of theophylline, pentobarbital and papaverine in tablets with a per cent standard deviation of 0.65 per cent, 0.75 per cent, and 0.65 per cent for each component has been described. This degree of reproducibility, comparable to that achieved by other methods, was obtained by adjusting solvent conditions to obtain absorbancy readings with maximum precision.

REFERENCES

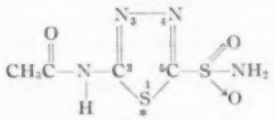
(1) Levi, L., and Hubley, C. E., *Anal. Chem.*, **28,** 1594 (1956).
(2) Butler, T. C., Ruth, J. M., and Tucker, G. F., Jr., *J. Am. Chem. Soc.*, **77,** 1486(1955).
(3) Fox, J. J., and Shugar, D., *Bull. soc. chim. Belg.*, **61,** 44(1952).
(4) Broughton, P. M. G., *Biochem. J.*, **63,** 207(1956).
(5) Goldbaum, L. R., *Anal. Chem.*, **24,** 1604(1952).
(6) Goldbaum, L. R., *J. Pharmacol. Exptl. Therap.* **94,** 68(1948).
(7) Maker, J. R., and Puckett, R. F., *J. Lab. Clin. Med.*, **45,** 896(1955).
(8) Mattson, L. N., THIS JOURNAL, **43,** 22(1954).
(9) McCallum, N. E. W., *J. Pharm. and Pharmacol.*, **6,** 733(1954).
(10) Mellon, M. G. "Analytical Absorption Spectroscopy," John Wiley and Sons, New York 1950, p. 369.
(11) Foster, G. E., and Macdonald, J., *J. Pharm. and Pharmacol.*, **3,** 127(1951).

The Synthesis of 1-S-35 Diamox*

By LEON CLARK and LLOYD J. ROTH

A procedure is described for the synthesis of S-35 labeled Diamox® (2-acetylamino-1,3,4-thiadiazole-5-sulfonamide), in relatively good yield from readily available starting materials.

DIAMOX is used in the treatment of congestive heart failure (1), glaucoma (2), and epilepsy (3). Since only minimal concentrations of drug are attained within certain body tissues, an isotopic label in the molecule provides a valuable tool for the study of its distribution pattern and metabolism in these tissues. The purpose of this report is to describe a procedure for the synthesis of Diamox with a sulfur-35 label in the heterocyclic ring.



1-S^{35} Diamox

EXPERIMENTAL[1]

S-35 Potassium Thiocyanate.—This intermediate is commercially available or may be conveniently

* Received February 25, 1957, from the Department of Pharmacology, University of Chicago, Chicago, Ill.
This work was aided by a research grant from the Multiple Sclerosis Foundation of America; also aided, in part, under a contract between the Atomic Energy Commission and The University of Chicago, by a research grant from the National Society for the Prevention of Blindness, and grant from the Wallace C. and Clara A. Abbott Memorial Fund.

[1] All melting points are uncorrected. Microchemical analyses were performed by William Saschek, of the Department of Chemistry, University of Chicago.

prepared from elemental sulfur-35 by a modification of the method of Sprinson and Rittenberg (4) for the C-14 labeled compound.

Sulfur-35 (25 mc.) was obtained from the Atomic Energy Commission in the form of a benzene solution (0.8 ml.). This solution was transferred to a tared 6-inch Pyrex test tube, which was then placed in a well-ventilated hood and allowed to remain, at room temperature, until the solvent had evaporated. The weight of the residue was found to be 8 mg. To it was added 32 mg. of nonisotopic sulfur and 100 mg. (1.5 mmoles) of potassium cyanide. The resultant mixture was fused at 310° for fifteen minutes in an atmosphere of nitrogen, then allowed to cool to room temperature. The crude S-35 potassium thiocyanate thus formed was used in the next step.

S-35 Thiosemicarbazide.—The synthesis of thiosemicarbazide from potassium thiocyanate and hydrazine sulfate has been described by Bambas (5). In a modification of this method, the above product was dissolved as completely as possible in 0.5 ml. of warm water, and to it was added 0.3 ml. of a stock solution of hydrazine sulfate.[2] The mixture was stirred thoroughly, then allowed to stand for thirty minutes at room temperature. At the end of this period, 5 ml. of methyl cellosolve was added, and the mixture was filtered. The filtrate was collected in a 6-inch Pyrex test tube.

The lower end of the test tube was surrounded by a preheated oil bath (150–179°)[3] and the filtrate was concentrated until its boiling point (liquid temperature) reached 130°. At this point, a cold-finger condenser was inserted into the open end of the test tube and heating was continued for two hours under reflux. At the end of this time, the solution was transferred, while still hot, to a small beaker, and was evaporated to dryness under a stream of nitrogen. The residue was recrystallized from boiling water to yield 69 mg. (0.75 mmole, 60%) of S-35 thiosemicarbazide; m. p. 179–181° (dec.). Reported m. p. 181–183° (dec.) (5).

1-S-35-2-Amino-1,3,4-thiadiazole-5-thiol.—Guha (6) has described the preparation of 2-amino-1,3,4-thiadiazole-5-thiol by the condensation of thiosemicarbazide and carbon disulfide in aqueous solvent. After a series of unsuccessful attempts to reproduce yields of the order of magnitude reported by this investigator, a search for a more efficient solvent for this condensation was begun. Of the solvents tested, pyridine was the most efficient and resulted in the best yields.

A mixture of S-35 thiosemicarbazide (69 mg., 0.75 mmole), carbon disulfide (1 ml.), and pyridine (7.5 ml.) was sealed in a heavy-walled Pyrex reaction tube and heated for four hours at 140–150°. At the end of this time, the tube and its contents were allowed to cool to room temperature, then chilled in an ice bath and opened. The contents were transferred to a small beaker, and the solvent was blown off under a stream of air. The residue was recrystallized from boiling water to yield 61.2 mg. (0.46 mmole, 61%) of 1-S-35-2-amino-1,3,4-thiadiazole-5-thiol, m. p. 237–242°. Reported m. p. 232°, 242° (6).

1-S-35-2-Acetylamino-1,3,4-thiadiazole-5-thiol.—The above product was acetylated in the customary manner (7) using 12.5 mg. of fused sodium acetate and 0.5 ml. of acetic anhydride. The crude 2-acetylamino-1,3,4-thiadiazole-5-thiol was dissolved in hot dilute ammonium hydroxide treated with charcoal and filtered. Neutralization of the filtrate with hydrochloric acid gave a precipitate of fine crystals, m. p. 297–300°. Reported m. p. 300° (dec.) (7). Yield 71.6 mg. (0.409 mmole, 89%).

1-S-35 Diamox.—The procedure of Roblin and Clapp (8) was found adaptable to the production of Diamox from the corresponding thiol on a scale of less than 1 millimole.

The above product was suspended in 5 ml. 33% acetic acid in a test tube. The suspension was cooled to 0° in an ice bath and saturated with chlorine gas, introduced by a finely drawn capillary. This operation required about ten minutes, during which time the temperature of the mixture was not allowed to exceed 8°. The sulfonyl chloride thus formed was collected on a sintered glass micro filter, washed with ice-cold water, and added, in small amounts, to liquid ammonia (15 ml.) in a beaker suspended in a slightly larger beaker. The excess ammonia was allowed to evaporate and the residue was purified in accordance with the procedure of Roblin and Clapp (8). Yield 51 mg. (0.23 mmole, 56%); m. p. 258–259°. Reported m. p. 258–259° (8).

Anal.—Calcd. for $C_4H_6N_4O_3S_2$: C, 21.6; H, 2.7; N, 25.2; S, 28.8. Found: C, 21.60; H, 2.77; N, 25.06; S, 28.59.

Radiochemical purity was established by means of paper chromatography, using a water-saturated butanol system. An autoradiogram of the chromatographic strip showed a single band of radioactivity.

Radiochemical yield: 4.5 millicuries (18% based on sulfur).

SUMMARY

1. Diamox with an S^{35} label in the heterocyclic ring has been synthesized on a one millimole scale in 18 per cent overall yield from elemental sulfur-35.

2. The essential feature of this synthesis is its economy with respect to time, labor, and cost of starting materials.

REFERENCES

(1) Belsky, H., *New Engl. J. Med.*, **249**, 140(1953).
(2) Becker, B., *Am. J. Ophthalmol.*, **38** (1:1), 109(1954).
(3) Merlis, S., *Neurology*, **4**, 863(1954).
(4) Sprinson, D. B., and Rittenberg, D., *J. Biol. Chem.*, **198**, 655(1952).
(5) Bambas, L. L., U. S. pat. 2,450,406, Oct. 5, 1948.
(6) Guha, P. C., *J. Am. Chem. Soc.*, **44**, 1510(1922).
(7) Freund, M., and Imgart, H., *Ber.*, **28** 946(1895).
(8) Roblin, R. O., Jr., and Clapp, J. W., *J. Am. Chem Soc.*, **72**, 4890(1950).

[2] This solution was prepared by dissolving 900 mg. of hydrazine hydrate (99–100%) and 2 gm. of hydrazine sulfate, $NH_2NH_2 \cdot H_2SO_4$ in a minimum amount of water and bringing the volume up to 3 ml.

[3] Because thiosemicarbazide is unstable at temperatures approaching its melting point, superheating of the reaction mixture can result in extensive decomposition of the product.

The Activity of β-Diethylaminoethyl Diphenylpropylacetate Hydrochloride on Ehrlich Ascites and Other Tumors*

By B. L. FREEDLANDER and F. A. FRENCH

β-Diethylaminoethyl diphenylpropylacetate hydrochloride (SKF 525A) inhibited the growth of Ehrlich ascites tumor and prolonged survival time in Swiss-Webster mice. The carcinostatic action was greatly decreased upon delay in therapy after tumor inoculation. The above drug was without inhibitory action on leukemias L-1210 and L-4946, adenocarcinoma 755, and neuroblastoma C-1300. SKF 525A did not augment the effect of several known carcinostatic agents.

BETA-DIETHYLAMINOETHYL DIPHENYLPROPYLACETATE HYDROCHLORIDE (SKF 525A)[1] has been studied extensively as a potentiating agent on the action of many drugs (1, 2). Its potentiating effect is largely one of slowing down the metabolism of certain drugs and hence prolonging their duration of action. SKF 525A blocks the degradation of diverse drugs by modifying the rate of a number of metabolic pathways. Metabolic steps which are modified include side chain oxidation, dealkylation, deamination, ether cleavage and hydroxylation. Consequently it was felt desirable to test SKF 525A on a variety of mouse tumors, by itself, and in combination with known chemotherapeutic agents.

METHODS

Female Swiss-Webster mice weighing 20–25 grams were inoculated with a 1:1 dilution of Ehrlich-Hauschka ES ascites fluid taken from six-day tumors. The number of cells used was approximately 10^6 in a 0.1-cc. inoculum. The drugs, in maximum tolerated doses, were given either intraperitoneally or orally by stomach tube once daily, starting one or three days following tumor inoculation, as indicated in Table I. The method used was similar to that described by Suguira (3). The mice were weighed initially and then at weekly intervals. An equal number of control mice were used in each experiment. Two main criteria were used as indices of activity: extension of survival time of treated mice over that of the controls, and the weight of the ascites fluid at a fixed time after initiation of the experiment. In the first type of experiment the 50% survival time was used as an index of drug effectiveness. This was determined by plotting cumulative death on a probability scale against time on a linear scale and fitting a straight line. In the second type of experiment the ascites fluid weight was determined at fourteen days according to the method described by Suguira and Creech (4). Nitrogen mustard (HN_2) was used as a positive control. For the other tumors, leukemia L-1210 and adenocarcinoma 755 in F_1-C57-dba hybrid mice, leukemia L-4946 in AKR mice, and neuroblastoma C-1300 in strain A mice, standard methods previously described were used (5).

RESULTS AND DISCUSSION

Results of the experiments are given in Tables I and II and Fig. 1. It is evident that the intraperitoneal route of administration was better than the oral route, and that a delay in therapy of three days nearly negates the effectiveness of SKF 525A. In experiments wherein therapy was started only one day after tumor inoculation there was an average of approximately twelve days' increase in 50% survival time over that of the controls; with therapy delayed three days the increase in survival time was insignificant. A measure of the spread in survival times, inversely proportional to the slope of the lines in Fig. 1, in a single experiment was

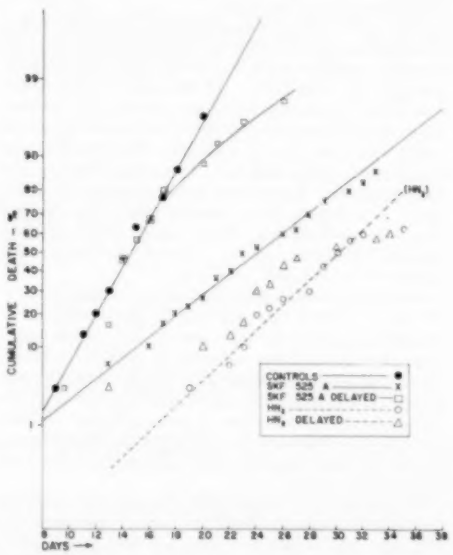


Fig. 1.—The comparative effect of early and delayed therapy of SKF 525A and HN_2 on the survival time of mice inoculated with ascites tumor.

* Received April 8, 1957, from the Research Laboratories, Mount Zion Hospital, San Francisco, Calif.
Supported in part by grants from the American Cancer Society and the Damon Runyon Memorial Fund for Cancer Research.
[1] SKF 525A was generously supplied by Smith, Kline and French Laboratories, Philadelphia, Pa.

TABLE I.—EFFECT OF SKF 525A ON INCREASED SURVIVAL TIME IN ASCITES TUMOR

Expt. No.	Drug	No. Mice	Dosage mg./Mouse Daily	Route	No. Treat.	Treat. Started Days After Tumor Inoc.	Mouse Wt. Incr. Gm. 10th Day	Days 50% Survival
I	SKF 525A	30	0.75	I.P.	6	1	0.6	24.1 ± 7.0[a]
	HN_2	30	0.01	I.P.	5	1	1.0	30.0 ± 5.7
	Controls	30	...	...	...	...	9.2	14.6 ± 3.0
II	SKF 525A	30	0.75	I.P.	6	3	5.9	14.6 ± 4.0
	HN_2	30	0.01	I.P.	5	3	3.2	30.0 ± 5.7
	Controls	30	...	...	...	...	9.2	14.6 ± 3.0
III	SKF 525A	20	0.75	I.P.	6	1	4.1	29.7 ± 10.8
	SKF 525A	20	1.5	Oral	7	1	10.2	17.7 ± 2.0
	Controls	20	...	...	...	...	10.7	15.6 ± 1.5

[a] The values given after the ± signs are not standard deviations but are the differences between the 84% and 50% survival times (or 50% and 16%) obtained from linear probability plots. This value is a measure of the average spread in survival time and is not a measure of deviation of survival time data from linearity.

TABLE II.—EFFECT OF SKF 525A ON WEIGHT OF ASCITES FLUID

Expt. No.	No. Mice	Dosage mg./Mouse Daily	Route	No. Treat.	Treat. Started Days After Tumor Inoc.	Mortality	Mouse Wt. Incr. 10th Day Gm.	Ascites Fluid Wt. 14th Day Gm.
I	20	0.75	I.P.	6	1	0	3.2	5.0 ± 1.2[a]
Controls	20	...	...	...	...	3	9.1	15.8 ± 1.2
II	7	0.5	I.P.	4	1	1	3.7	9.0[b]
Controls	8	...	...	...	...	2	5.9	15.3
III	20	3.0	Oral	6	1	2	4.1	11.8 ± 0.8
Controls	20	...	...	...	...	3	9.1	15.8 ± 1.2

[a] In experiments I and III the values after the ± signs are standard deviations of the average ascites fluid weights computed on the basis of accepted small sample formulas.
[b] Because of the small number of animals involved in Experiment II no deviations were computed.

±3.0 days for the controls, and ±7.0 days for the treated animals. In contrast to SKF 525A, HN_2 was as effective by delayed therapy as it was on early administration. The results of therapy with SKF 525A by the two different indices of evaluation, namely survival (Table I) and fluid weight (Table II) are qualitatively in accord. Smears of the ascites tumor suspension from the treated mice, taken on the eighth day after inoculation and stained with Papanicolaou stain, showed very few tumor cells, which were almost all degenerated; smears from the control mice taken at the same time showed an abundance of tumor cells.

Figure 1 gives a graphic comparison of results relative to time for early and delayed therapy by SKF 525A. It may be noted that in the delayed therapy group treated with SKF 525A no significant effect is discernible until over 70% mortality is obtained. Then a significant straggling and delayed mortality can be seen. The average deviation of individual points from the straight lines, in days, may be taken as a measure of the appropriateness of this graphical mode of representation. The value of the scatter for the control curve is about ±0.2 day and for the group which received early treatment the average scatter is about ±0.6 day. The lowest line on Fig. 1 shows the results obtained with HN_2. A straight line gives only a fair representation of the data and there is a marked curvature and change of slope at about the 50% mortality region. It may also be noted that, in contrast with SKF 525A, the effects of prompt and delayed treatment with HN_2 are not significantly different.

SKF 525A was found negative against leukemia L-4946, leukemia L-1210, neuroblastoma C-1300 and adenocarcinoma 755. Additional experiments were performed using combined treatment with SKF 525A and known chemotherapeutic agents. No additive or synergistic effects were noted on the above tumors treated with SKF 525A combined with HN_2, 6-mercaptopurine, urethane, amethopterin, or 8-azaguanine.

Experiments on this drug were originally performed with the expectation of finding potentiation effects. These did not materialize. Instead, a highly specific action on the Ehrlich ascites tumor was found. The mechanism of this action is not known but may be ascribed, tentatively, to an altered oxidative metabolism. Further work is in progress on several related compounds and on combined action of SKF 525A with other agents.

REFERENCES

(1) Axelrod, J., Reichenthal, J., and Brodie, B. B., *J. Pharmacol. Exptl. Therap.*, **112**, 49(1954).
(2) Axelrod, J., *J. Biol. Chem.*, **214**, 753(1955).
(3) Suguira, K., *Cancer Research*, **13**, 431(1955).
(4) Suguira, K., and Creech, H. J., *Ann. N. Y. Acad. Sci.*, **63**, 962(1956).
(5) Cancer Chemotherapy National Service Center, "Specifications for Screening of Chemical Agents Against Animal Tumors," 1957.

The Effect of Various Sugars and Polyols on the Stability of Vitamin B_{12} *

By MARTIN BARR, S. ROBERT KOHN, and LINWOOD F. TICE

The stability of vitamin B_{12} was studied in aqueous solutions of sorbitol, glycerin, dextrose, and sucrose. Solutions were kept at 25°, 45°, and 100° for periods up to one hundred and forty days. Vitamin B_{12} content was determined spectrophotometrically using an absorption maximum of 361 mμ. It was found that vitamin B_{12} is compatible with sorbitol and glycerin at 25° and 45°. Dextrose and sucrose solutions were found to cause the loss of vitamin B_{12} in solution. Dextrose was more destructive to the vitamin than was sucrose.

SORBITOL, GLYCERIN, dextrose, and sucrose have been used in oral liquid preparations containing vitamin B_{12}. It has been reported that sorbitol and glycerin are compatible with vitamin B_{12}, while reducing agents such as dextrose and sucrose (after hydrolysis) are not (1,2). However, there is little information available indicating the degree to which dextrose and sucrose cause the loss of vitamin B_{12}. This led the authors to carry out studies on the stability of vitamin B_{12} in solutions containing sorbitol, glycerin, dextrose, and sucrose over a period of time at different temperatures.

EXPERIMENTAL

Sterile solutions containing approximately 30 mcg. of vitamin B_{12} in 50% w/v solutions of sorbitol,[1] glycerin,[2] dextrose,[3] and sucrose[4] were prepared in the following manner.

One hundred grams of sorbitol, glycerin, dextrose, and sucrose were dissolved in sufficient purified water to make 1,985 ml., using a 2000-ml. volumetric flask. The solutions were then sterilized in an autoclave by heating at 15 pounds pressure (121°) for twenty minutes. A stock solution containing 60 mg. of vitamin B_{12}[5] in purified water was prepared and sterilized by passing through a Selas filter.[6] Fifteen milliliters of the stock vitamin B_{12} solution were then transferred aseptically with a sterile pipet into each of the 2,000-ml. volumetric flasks containing the polyol or sugar solutions. The finished volumes of these solutions were then 2,000 ml. and they contained approximately 30 mcg. of vitamin B_{12} per ml. Ten-milliliter portions of each solution were then transferred into 10-ml. glass ampuls using a sterile, automatic filler.[7] Sterile solutions containing only vitamin B_{12}, sorbitol, glycerin, dextrose, and sucrose in purified water were also prepared and transferred into 10-ml. glass ampuls. These solutions served as blanks and controls in the study. The ampuls were sealed and a representative number of each solution was tested for sterility using a fluid thioglycollate medium. All were found to be sterile.

The effects of the polyols and sugars on the stability of vitamin B_{12} were studied at three different temperatures. One series was maintained at 100° in an oven for forty-eight hours. A second series was kept at 45°, using a thermostat-controlled room for this purpose. A third series was maintained at room temperature (approximately 25°). The solutions which were studied at 45° and 25° were observed for a total of one hundred and forty days. Enough ampuls containing the solutions are available to permit the continuation of this study for at least one year.

The solutions in the ampuls were assayed periodically in order to determine the extent of deterioration of the vitamin B_{12} in the presence of the polyols and sugars. The solutions were assayed for vitamin B_{12} content using the spectrophotometric assay in U. S. P. XV (3), which is based on an absorption maximum at 361 mμ. The sorbitol, glycerin, dextrose, and sucrose solutions were used as blanks and the vitamin B_{12} solution was used as a control. All solutions for which a vitamin B_{12} content is reported were found to exhibit ultraviolet absorption characteristics typical of the vitamin (3).

The results of the stability studies are reported in Tables I and II, and in Figs. 1 and 2.

RESULTS AND DISCUSSION

The results indicate that, under accelerated test conditions of 100°, there was a marked loss in vitamin B_{12} content in all solutions containing the vitamin.

There was a loss of nearly 70% in vitamin content in the dextrose solutions in eight hours and a deterioration of nearly 40% in the sucrose solutions in the same period of time. It was not possible to determine the vitamin content in the dextrose and sucrose solutions after eight hours because of discoloration of the solutions due to caramelization. It was found that these solutions no longer exhibited the ultraviolet absorption characteristics of vitamin B_{12}.

It is interesting to note that the loss of vitamin B_{12} in 50% w/v sorbitol and glycerin solutions was not as great as in purified water alone at 100°. This

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Presented to the Scientific Section, A.PH.A., New York meeting, May 1957.

[1] Crystalline D-Sorbitol, Atlas Powder Co.
[2] Glycerin, U. S. P. XV, Colgate-Palmolive-Peet Co.
[3] Dextrose, U. S. P. XV, New York Quinine and Chemical Works, Inc.
[4] Sucrose, Analytical Reagent, J. T. Baker Chemical Co.
[5] Crystalline Vitamin B_{12}, Merck and Co., Inc.
[6] 03 Selas Filter, Selas Corporation of America.
[7] Ampul Filling Machine, Baltimore Biological Laboratory, Inc., Baltimore, Md.

TABLE I.—EFFECTS OF POLYOLS AND SUGARS[a] ON VITAMIN B_{12} (INITIAL CONCENTRATION—29.4 MCG./ML.)

Storage at 25°, days →	10	17	24	31	45	63	84	112	140
Sorbitol	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4
Glycerin	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4
Dextrose	28.9	28.5	27.7	27.3	26.7	25.8	24.4	23.4	22.1
Sucrose	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4
Purified water	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4
Storage at 45°, days →	**10**	**17**	**24**	**31**	**45**	**63**	**84**	**112**	**140**
Sorbitol	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4
Glycerin	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4
Dextrose	28.0	27.1	26.1	25.1	23.9	22.0	19.7	17.4	[b]
Sucrose	28.5	28.0	27.3	27.0	25.0	24.7	23.1	21.4	[b]
Purified water	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4	29.4
Storage at 100°, hours →	**2**	**4**	**8**	**16**	**24**	**48**			
Sorbitol	29.3	29.0	28.5	27.6	26.8	24.9			
Glycerin	29.1	28.5	27.9	26.4	25.5	22.7			
Dextrose	23.7	16.4	9.1	[b]	[b]	[b]			
Sucrose	25.2	22.4	17.9	[b]	[b]	[b]			
Purified water	29.0	28.4	27.2	25.1	22.9	19.0			

[a] Polyols and sugars are present in a concentration of 50% w/v.
[b] It was impossible to analyze these solutions as they were discolored due to the caramelization of the sugars.

TABLE II.—LOSS, IN %, OF VITAMIN B_{12} CONTENT IN SOLUTIONS CONTAINING VARIOUS POLYOLS AND SUGARS[a] (INITIAL CONCENTRATION—29.4 MCG./ML. VITAMIN B_{12})

Storage at 25°, days →	10	17	24	31	45	63	84	112	140
Sorbitol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glycerin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dextrose	1.7	3.1	5.8	7.1	9.2	12.3	17.0	20.4	24.8
Sucrose	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Purified water	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Storage at 45°, days →	**10**	**17**	**24**	**31**	**45**	**63**	**84**	**112**	**140**
Sorbitol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glycerin	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dextrose	4.8	7.8	11.2	14.6	18.7	25.2	33.0	40.8	[b]
Sucrose	3.1	4.7	7.1	8.2	11.9	16.0	21.4	28.2	[b]
Purified water	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Storage at 100°, hours →	**2**	**4**	**8**	**16**	**24**	**48**			
Sorbitol	0.3	1.4	2.7	6.1	8.5	15.8			
Glycerin	1.0	3.1	5.1	10.2	13.3	22.4			
Dextrose	19.3	44.4	69.0	[b]	[b]	[b]			
Sucrose	14.0	23.8	39.1	[b]	[b]	[b]			
Purified water	1.4	3.3	7.5	14.6	22.1	35.2			

[a] Polyols and sugars are present in concentrations of 50% w/v.
[b] It was impossible to analyze these solutions as they were discolored due to the caramelization of the sugars.

may be due to the fact that there is a lower concentration of water in these solutions which reduces the rate of hydrolysis, which is known to be involved in the deterioration of vitamin B_{12}.

Dextrose and sucrose were the only substances which caused a reduction in vitamin B_{12} content at 45°. There was a reduction of about 40% caused by dextrose in one hundred and twelve days and a loss of nearly 30% in the sucrose solutions during the same period of time. It was not possible to determine the vitamin B_{12} content of the dextrose and sucrose solutions stored at 45° for one hundred and forty days because of the decided discoloration of the solutions due to caramelization. These solutions no longer exhibited the ultraviolet absorption characteristics of vitamin B_{12}.

Dextrose was the only substance which produced a loss in vitamin content in one hundred and forty days at 25°. The deterioration at this temperature was slow, but it did amount to about a 25% loss in vitamin content during this time.

Spot microbiological determinations for vitamin B_{12} activity were carried out on a number of the vitamin solutions stored at 25° and 45°. The results revealed that only dextrose and sucrose caused a reduction in vitamin B_{12} activity. In this respect, the results were in general agreement with those obtained by the spectrophotometric method.

The pH of the vitamin B_{12} solutions was between 5.2-6.5. All solutions became more acid during storage. Those showing the greatest loss in vitamin content were also found to be reduced the most in pH. For example, the pH of the sucrose-vitamin B_{12} solutions initially was 6.5 but, after storage at 25° and 45°, their pH values were 4.3 and 3.6, respectively. The initial pH of sorbitol-vitamin B_{12}

solutions was 5.2, falling to 4.5 and 4.2 after storage at 25° and 45°, respectively. In this latter instance, the drop in pH was not accompanied by a loss in B_{12} content.

CONCLUSIONS

Based on one hundred and forty days of observation, it is possible to reach certain conclusions concerning the stability of vitamin B_{12} in the presence of various polyols and sugars. Dextrose is a reducing agent and sucrose, after hydrolysis, becomes one.

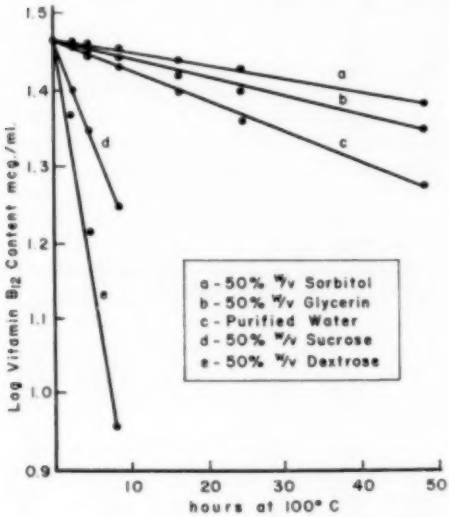

Fig. 1.—Effect of various polyols and sugars on B_{12} at 100°.

Both sugars caused a reduction in the vitamin B_{12} content of solutions. In this respect, the results reported here are in agreement with those of earlier workers (1, 2). Dextrose was more active in reducing the vitamin B_{12} content in the solution than sucrose for the probable reason that the latter must first undergo hydrolysis. Sorbitol and glycerin did not produce a loss in vitamin content except at a temperature of 100°. At this temperature, although there was a reduction of vitamin B_{12} content in sorbitol and glycerin solutions, it was less than the loss observed in the aqueous control. On the basis of

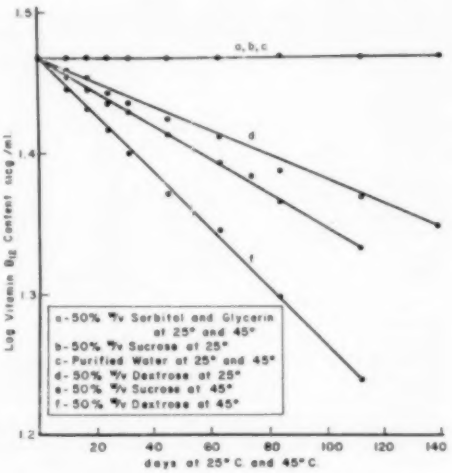
Log Vitamin B12 Content mcg./ml.
days at 25° C. and 45° C.
a-50% %v Sorbitol and Glycerin at 25° and 45°
b-50% %v Sucrose at 25°
c-Purified Water at 25° and 45°
d-50% %v Dextrose at 25°
e-50% %v Sucrose at 45°
f-50% %v Dextrose at 45°

Fig. 2.—Effect of various polyols and sugars on B_{12} at 25° and 45°.

one hundred and forty days of study, it appears that both sorbitol and glycerin are compatible with vitamin B_{12}.

SUMMARY

1. Studies have been carried out for a period of one hundred and forty days on the stability of vitamin B_{12} in sorbitol, glycerin, dextrose, and sucrose solutions.

2. Vitamin B_{12} was found to be compatible in sorbitol and glycerin solutions at 25° and 45°.

3. Dextrose and sucrose were found to cause the decomposition of vitamin B_{12} in solution at 25° and 45°. Dextrose was more destructive in its effect than sucrose.

REFERENCES

(1) "Vitamin B_{12}," Part I, Merck Service Bulletin, Merck & Co., Inc., Rahway, N. J., 1953, pp. 8–9.
(2) Gakenheimer, W. C., *Drug & Cosmetic Ind.*, **72**, 320 (1953).
(3) United States Pharmacopoeia, Fifteenth Revision, Mack Printing Co., Easton, Pa., 1955, pp. 186–187.

Steroidal Sapogenins XLIII.*

Survey of Plants for Steroidal Sapogenins and Other Constituents

By MONROE E. WALL, C. S. FENSKE, H. E. KENNEY, J. J. WILLAMAN, D. S. CORRELL, B. G. SCHUBERT, and H. S. GENTRY

This is a report of the chemical examination of the fourth 1,000 accessions in a survey of plants for steroidal sapogenins and is thus a continuation of the reports on the first three 1,000 accessions (1–6). Data are given for 950 samples, representing 100 families, 272 genera, and 432 identified species. Of these, 14 families, 151 genera, and 376 species are new in this series. Quantitative data are given for the occurrence of 15 steroidal sapogenins, of which two, gentrogenin and correllogenin, are new. Qualitative data are given for the occurrence of these groups of constituents: saponins, flavonoids, alkaloids, tannins, and unsaturated sterols. There is no previously published chemical information on about 50 per cent of the species examined.

THIS IS A CONTINUATION of the previous reports (1–6) on the first three 1,000 accessions. It covers the fourth 1,000 plant samples in our survey of the plant world for steroidal sapogenins, and contains qualitative results for some other constituents. In the collections, emphasis was again placed on species of *Agave*, *Dioscorea*, and *Yucca*, partly because these genera are still the best sources of the steroidal sapogenins, and partly because we have aimed to make the coverage of these three as complete as possible. As a result, *Yucca* is practically complete; *Agave* is about 60 per cent complete, with 160 reasonably available species covered; *Dioscorea* is rather complete as to North and Central America, but the 53 species covered are not more than 9 per cent of the total in this world-wide genus.

PROCUREMENT

In continuation of the policy established earlier in this program, concentrated collecting was carried on for certain genera—*Dioscorea*, *Agave*, and *Yucca*—particularly in Mexico, but also in Central and South America. Other collecting was concentrated on particular geographical areas rather than individual plant-groups. Among these regions are included Cuba and the Virgin Islands, Chile and Peru, Turkey, South Africa, and the southeastern United States. Large collections of some genera under cultivation at the Plant Introduction Gardens at Glenn Dale, Md., and Coconut Grove, Fla., were also supplied for testing. In all, 25 collectors supplied representatives of 101 families included in the 1,000 samples analyzed.

METHODS

Since many of the sapogenin assays were made on repeat collections, we felt that a procedure somewhat less precise but more rapid than the previously described methods (1, 10) would suffice.

A crude sapogenin sample was obtained by acid hydrolysis and alkali purification as described previously (10). The total crude sample was dissolved in chloroform. An aliquot equivalent to approximately 0.1 gram was taken, evaporated to dryness in a tared beaker and the residue accurately weighed. From the value obtained, the total dry weight of crude sapogenin could be obtained. The residue was then acetylated, dried, and the weight of crude acetate obtained. An infrared determination was then carried out and the percentage of steroidal sapogenin calculated as described previously (10–12).

The individual sapogenins were separated and detected by paper chromatography. For this purpose two systems were used. In both systems approximately 500 micrograms of crude sapogenin (free hydroxyl forms) were placed on Whatman No. 4 paper saturated with an immobile phase. For monohydroxy, nonketonic sapogenins the immobile phase was phenylcellosolve and the moving phase a mixture of 98 parts benzene and 2 parts methanol. In the case of ketonic or dihydroxy sapogenins the fixed phase consisted of propylene glycol and the mobile phase a mixture of 80 parts benzene, 15 parts cyclohexane, and 5 parts methanol. The color developing agent was an ethanolic solution of phosphomolybdic acid.

By using a combination of infrared techniques and paper chromatography the sapogenins could be separated, identified, and listed as to major or minor constituent.

RESULTS

A compilation of all data is given in Table I, page 654, *et seq.* A summary of the steroidal sapogenins found in this series is given in Table II, page 683, *et seq.*

* Received July 24, 1957, from the Eastern Regional Research Laboratory, a laboratory of the Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture, Philadelphia 18, Pa., and the Crops Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Md.

In order to expedite the examination of such a large number of plant materials, groups of workers were organized, each group handling certain phases of the whole procedure. We gratefully acknowledge the work of J. W. Garvin, Walter Rumph, R. A. Pierce, H. M. Neilson, G. H. Eppley, Theodore Perlstein, H. W. Jones, Samuel Serota, H. A. Walens, and C. R. Eddy.

TABLE I.—SAPOGENINS AND OTHER CONSTITUENTS FOUND IN THE PLANT COLLECTIONS

Code for Sapogenins (the Suffix "Genia" Omitted)

c—chloro
co—corrello
d—dios
dh—9-dehydroheco
dm—9-dehydromano
g—gito
ge—gentro
h—heco
k—kammo
m—mano
mk—marko
p—penno
sa—samo
sm—smila
ss—sarsasapo
t—tigo
y—yucca
ya—yamo

Code for Plant Parts

b—bark
bu—bulb
fl—inflorescence
fr—fruit
ib—inflorescent bud
l—leaf
r—root
rh—rhizome
s—stem
sd—seed
t—tuber
tw—twig
w—whole plant above ground
wd—wood
y—young

Code for Source Names

C. G. F.—U. S. Plant Introduction Garden, Coconut Grove, Fla. (cult.)
Chico, Cal.—U. S. Plant Introduction Garden, Chico, Cal. (cult.)
G. D. M.—U. S. Plant Introduction Garden, Glenn Dale, Md. (cult.)
Hunt. Bot. Gard.—Huntington Botanic Garden, Pasadena, Cal. (cult.)
Mayaguez, P.R.—Agricultural Experiment Station, U. S. Dept. Agriculture (cult.)

Other Codes

ir—infrared analysis
mi—micro screening test
ni—not identified
nss—not sufficient sample
tr—trace

Accession No.	Collection: Species	Collection: Source	Collection: Date Collected	Collection: Plant Part	Hemolysis Test	Sapogenins: Total, M. F. B., %	Sapogenins: Identified, % of Total	Flavonoids	Alkaloids	Tannins	Sterols
				ACANTHACEAE							
3975	*Aphelandra jacobinioides*	Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	++
3658	*Brillantaisia lamium*	C. G. F.	3/54	l, s	−	...	.....	0	0	0	+
3859	*Mendoncia pilosa*	Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	+	+++
3959	*Mendoncia pilosa*	Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	+	++
3951	*Sanchezia pennellii*	Tingo Maria, Peru	4/54	l, s	−	...	.....	+	0	0	+
				ACERACEAE							
3835	*Acer negundo*	Wake Co., N. C.	4/54	tw, l	+	0	.....	0	0	0	++
3933	*Acer negundo*	Clarke Co., Ga.	4/54	l, s	−	...	.....	0	0	0	+++
3731	*Acer rubrum*	Southport, Brunswick Co., N. C.	4/54	fr	−	...	.....	++	0	+++	++
3918	*Acer rubrum*	Southport, Brunswick Co., N. C.	4/54	l	−	...	.....	0	0	0	+++
				AMARANTHACEAE							
3322	*Chamissoa sp.*	Mexico City, Mex.	1/53	sd	−	...	.....	0	0	0	0
3323	*Chamissoa sp.*	Mexico City, Mex.	1/53	fl	−	...	.....	0	+	0	+
3329	*Chamissoa sp.*	Mexico City, Mex.	1/53	l	+	0	.....	0	0	0	0
3848	*Chamissoa sp.*	Tingo Maria, Peru	4/54	l, tw	−	...	.....	0	0	0	++
3880	*Chamissoa sp.*	Carmen Alto, Km. 177, Tingo Maria, Peru	4/54	l, s	+	0	.....	0	0	0	++
3985	*Cyathula achyranthoides*	Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	0
				AMARYLLIDACEAE							
3019	*Agave angustiarum*	W. of Iguala, Guer., Mex.	8/52	l	+	0.2	d100	..	..	..	..
3040	*Agave angustiarum*	Canyon Iguala, Guer., Mex.	9/52	l	+	0	.....	..	..	..	..
3142	*Agave cf. angustiarum*	Camaron, Oax., Mex.	9/52	l	+	0	.....	..	..	..	..

3076	*Agave angustifolia*	N. E. of Cd. Oaxaca, Oax., Mex.	9/52	1		+	0	.....	..	..	..	..
3128	*Agave angustifolia*	Istmo Tehuantepec, Oax., Mex.	9/52	1		+	0.7	h100	..	..	..	..
3491	*Agave applanata*	Zacatepec, Pueb., Mex.	4/53	1		+	0	.....	..	..	..	..
3500	*Agave applanata*	Tulancingo, Hid., Mex.	5/53	1		+	0.2 ir	h; t, m	..	..	..	..
3130	*Agave atrovirens*	S. Cristobal Casas, Chia., Mex.	9/52	1		+	0	.....	..	..	..	..
3207	*Agave atrovirens*	Cholula, Pueb. Mex.	10/52	1		+	0	.....	..	..	..	..
3282	*Agave aurea*	N. of Comondu, B. C., Mex.	11/50	1	dead	+	0.3 ir	h; dh	..	..	..	..
3283	*Agave aurea*	N. of San Xavier, B. C., Mex.	12/52	1	green	+	1.2 ir	h; dh	..	..	..	..
3293	*Agave aurea*	N. of Comondu, B. C., Mex.	11/52	1		+	0.1 ir	h; dh, m, dm	..	..	..	..
3304	*Agave aurea*	N. of Comondu, B. C., Mex.	11/52	1		+	1.4 ir	h; t, m, dm	..	..	..	..
3294	*Agave aurea X A. sobria*	N. of Comondu, B. C., Mex.	11/52	1		+	0.7 ir	h; t, m, dm	..	..	..	..
3246	*Agave brandegeei*	S. of LaPaz, B. C., Mex.	11/52	1		+	1.2 ir	h; dh, t, g, m, dm	..	..	..	..
3257	*Agave brandegeei*	Cabo San Lucas, B. C., Mex.	11/52	1		+	0	.....	..	..	..	..
3261	*Agave brandegeei*	S. of LaPaz, B. C., Mex.	11/52	1		+	1.1 ir	h; dh	..	..	..	..
3281	*Agave brandegeei*	N. of Todos Santos, B. C., Mex.	11/52	1		+	0	.....	..	..	..	..
3295	*Agave brandegeei*	N. of Todos Santos, B. C., Mex.	11/52	1		+	0.7 ir	h, m	..	..	..	..
3302	*Agave brandegeei*	S. of Todos Santos, B. C., Mex.	11/52	1		+	1.3 ir	t; h	..	..	..	..
3311	*Agave brandegeei*	Cabo San Lucas, B. C., Mex.	11/52	1		+	3.6	t; h, m, dm	..	..	..	..
3250	*Agave cerulata*	Calmalli, B. C., Mex.	12/52	1		+	1.1 ir	h; t, g, m, dm	..	..	..	..
3269	*Agave cerulata*	Calmalli, B. C., Mex.	12/52	1		+	0.5 ir	h; t, m, dm	..	..	..	..
3273	*Agave cerulata*	N. W. of Laguna Seca Chapala, B. C., Mex.	12/52	1		+	0.4	h50; m, dm50	..	..	..	..
3284	*Agave cerulata*	Calmalli, B. C., Mex.	12/52	1	dead	+	1.2 ir	h; dh, m				
3288	*Agave cerulata*	N. W. of Laguna Seca Chapala, B. C. Mex.	12/52	1		+	1.0 ir	m, h, g; t, dm	..	..	..	..
3289	*Agave cerulata*	N. W. of Laguna Seca Chapala, B. C., Mex.	12/52	1	dead	+	0.5 ir	h; dh, m, dm	..	..	..	..
3303	*Agave cerulata*	E. of San Ignacio; B. C., Mex.	12/52	1	dead	+	2.0 ir	h; m	..	..	..	..
3308	*Agave cerulata*	N. W. of Laguna Seca Chapala, B. C., Mex.	12/52	1		+	3.4	h; dh, m, dm	..	..	..	..
3310	*Agave cerulata*	San Ignacio, B. C., Mex.	12/52	1		+	1.5 ir	h; t, m, dm	..	..	..	..
3024	*Agave collina*	N. of Taxco, Guer., Mex.	8/52	1		+	0	.....	..	..	..	..
3139	*Agave collina*	N. of Tehuantepec, Caxaca, Mex.	9/52	1		+	0	.....	..	..	..	..
3041	*Aff. collina*	Pachina, Guer., Mex.	9/52	1		+	0	.....	..	..	..	..
3341	*Agave desertii*	Pinyon Flats, Sta., Rosa Mt., Cal.	1/53	1		+	0.6 ir	h; m, dm, g	..	..	..	..
3193	*Agave deweyana*	Manuel, Tam., Mex.	10/52	1		+	0.1 ir	t; h, m	..	..	..	..
3099	*Agave ferox*	Tejupan, Oax., Mex.	9/52	1		+	0	.....	..	..	..	..
3506	*Agave ferox*	Tejupan, Oax., Mex.	5/53	1		+	0.4 ir	h; m, dm, t	..	..	..	..
3162	*Agave fourcroydes*	N. of Tehuantepec, Oax., Mex.	9/52	1		+	0	.....	..	..	..	..
3189	*Agave fourcroydes*	Manuel, Tam., Mex.	10/52	1	pulp	−	...	.....	..	..	..	..
3438	*Agave fourcroydes*	San Andres, El Salv.	3/53	1		+	0	.....	..	..	..	..
3482	*Agave fourcroydes*	La Casa Garza, Tam., Mex.	5/53	1		+	0.3 ir	t; g, m	..	..	..	..

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis Test	Sapogenins: Total, M. F. B., %	Sapogenins: Identified, % of Total	Flavonoids	Alkaloids	Tannins	Sterols
				AMARYLLIDACEAE							
3079	*Agave aff. fourcroydes*	N. of Miahautlan, Oax., Mex.	9/52	l	+	0	.....	..	..	..	..
3192	*Agave funkiana*	Jacala, Hid., Mex.	10/52	l	+	0.6 ir	t; m	..	..	..	..
3194	*Agave cf. funkiana*	Jacala, Hid., Mex.	10/52	l	+	0.6	sm85; y15	..	..	..	..
3286	*Agave goldmaniana*	N. W. of Mesquital, B. C., Mex.	12/52	l dead	+	0.7 ir	m; dm, h, dh	..	..	..	..
3259	*Agave goldmaniana*	Punta Prieta, B. C., Mex.	12/52	l	+	0.6 ir	h; dh	..	..	..	..
3256	*Agave goldmaniana*	Punta Prieta, B. C., Mex.	12/52	l dead	+	0.8 ir	m; dm, t	..	..	..	..
3285	*Agave goldmaniana*	Punta Prieta, B. C., Mex.	12/52	l	+	0.9 ir	h; m, dm	..	..	..	..
3039	*Agave horrida*	Taxco, Guer., Mex.	9/52	l	+	0	.....	..	..	..	..
3042	*Agave horrida*	Cuernavaca, Morel., Mex.	9/52	l	+	0	.....	..	..	..	..
3043	*Agave horrida*	Cuernavaca, Morel., Mex.	9/52	l	+	0	.....	..	..	..	..
3047	*Agave horrida*	Cuernavaca, Morel., Mex.	9/52	l	+	0	.....	..	..	..	..
3131	*Agave aff. horrida*	San Mateo Cajones, Oax., Mex.	10/52	l	+	0	.....	..	..	..	..
3163	*Agave aff. horrida*	Zanatepec, Oax., Mex.	9/52	l	+	0	.....	..	..	..	..
3038	*Agave intrepida*	Tepoztlan, Morel., Mex.	9/52	l	+	0	.....	..	..	..	..
3154	*Agave kerchovei*	Huajuapan, Oax., Mex.	10/52	fl	+	0	.....	..	..	..	..
3396	*Agave letonae*	Zamorano, Hond.	2/53	l	+	0.2 ir	t; h	..	..	..	..
3196	*Agave lophantha*	Mantle, Tam., Mex.	10/52	l	+	0.4 ir	t; h	..	..	..	..
3206	*Agave macroacantha*	Acultzingan, Ver., Mex.	10/52	l	−	...	.....	..	..	..	..
3204	*Agave mapisaga*	Lagunilla, Hid., Mex.	10/52	fr	−	...	.....	..	..	..	..
3205	*Agave mapisaga*	Cholula, Pueb., Mex.	10/52	l	+	0.06 ir	h; t, dh	..	..	..	..
3074	*Agave marmorata*	Miahuatlan, Oax., Mex.	9/52	l	+	0.15	sm35; c65	..	..	..	..
3105	*Agave marmorata*	Tehuitzingo and Acatlan, Pueb., Mex.	9/52	l	+	0.6	sm100	..	..	..	..
3121	*Agave marmorata*	S. of Acatlan, Pueb., Mex.	9/52	l	+	0	.....	..	..	..	..
3158	*Agave mescal*	S. Christobal Casas, Chia., Mex.	9/52	l	+	0	.....	..	..	..	..
3160	*Agave mescal*	S. Christobal Casas, Chia., Mex.	9/52	l	+	0	.....	..	..	..	..
3490	*Agave mirabilis*	Jalapa, Ver., Mex.	4/53	l	+	0.6 ir	h; t, g, m, dm	..	..	..	..
3208	*Agave mitraeformis*	Tehuacan, Pueb., Mex.	10/52	fr	+	0.3 ir	h; dh, g	..	..	..	..
2247	*Agave nelsonii*	San Fernando, B. C., Mex.	12/52	l	+	1.3	m80; dm20	..	..	..	..
3248	*Agave nelsonii*	San Fernando, B. C., Mex.	12/52	l	+	2.5 ir	m; dm, h, t	..	..	..	..
3249	*Agave nelsonii*	San Fernando, B. C., Mex.	12/52	l	+	0.6	m, dm	..	..	..	..
3260	*Agave nelsonii*	San Fernando, B. C., Mex.	12/52	l	+	2.2 ir	t, m; h, dm	..	..	..	..
3272	*Agave nelsonii*	San Fernando, B. C., Mex.	12/52	l	+	1.3 ir	h; t, m, dm	..	..	..	..
3298	*Agave nelsonii*	San Fernando, B. C., Mex.	12/52	l dead	+	1.0 ir	t, m; h, dh, dm	..	..	..	..
3514	*Agave nelsonii*	San Fernando, B. C., Mex.	5/53	l	+	1.1 ir	m, dm, t; g, h	..	..	..	..
3104	*Agave pampaniaiana*	Nochistlan, Oax., Mex.	9/52	l	+	0.4	h100	..	..	..	..
3149	*Agave polyscantha*	Zacatepec, Mixes, Oax., Mex.	9/52	l	+	0	.....	..	..	..	..
3195	*Agave polyscantha*	Jacala, Hid., Mex.	10/52	l	+	0	.....	..	..	..	..
3509	*Agave portoricensis*	Maricao Forest, P. R.	5/53	l	+	0.5 mi	.....	..	..	..	..
3078	*Agave potatorum*	N. E. of Cd. Oaxaca, Mex.	9/52	l	+	0	.....	..	..	..	..
3156	*Agave potatorum*	Tuxtla Gutierrez, Chia., Mex.	9/52	l	+	0	....	..	..	..	..

3233	*Agave promontorii*	Balboa Park, San Diego, Cal.	11/52	1	+	0	.....	..	..	..	..
3245	*Agave promontorii*	Balboa Park, San Diego, Cal.	12/52	lb	+	0	.....	..	..	..	..
3252	*Agave promontorii*	W. base of Sierra Laguna, B. C., Mex.	11/52	1	+	0.4 ir	t; m	..	..	..	..
3262	*Agave promontorii*	W. base of Sierra Laguna, B. C., Mex.	11/52	1	+	3.4 ir	h; dh, m, t	..	..	..	..
3266	*Agave promontorii*	Rancho Burrera, Sierra Laguna, B. C., Mex.	11/52	lb	+	0.2 ir	h; t	..	..	..	..
3270	*Agave promontorii*	W. summit of Sierra Laguna, B. C., Mex.	11/52	1	+	0.8 ir	h; m, dm	..	..	..	..
3275	*Agave promontorii*	W. Summit of Sierra Laguna, B. C., Mex.	11/52	1	+	0.4 ir	h; dh	..	..	..	..
3287	*Agave promontorii*	W. summit of Sierra Laguna, B. C., Mex.	11/52	1	+	0.7 ir	h; t, dh	..	..	..	..
3291	*Agave promontorii*	W. summit of Sierra Laguna, B. C., Mex.	11/52	1 dead	+	0.9 ir	t; m	..	..	..	..
3296	*Agave promontorii*	W. summit of Sierra Laguna, B. C., Mex.	11/52	1	+	0.9 ir	h; m, dh, dm	..	..	..	..
3299	*Agave promortorii*	W. summit of Sierra Laguna, B. C., Mex.	11/52	1	+	0.5 ir	h; dh	..	..	..	..
3309	*Agave promontorii*	W. summit of Sierra Laguna, B. C., Mex.	11/52	1	+	0.8 ir	h; t, g, m	..	..	..	..
3331	*Agave promontorii*	Sierra Laguna, B. C., Mex.	11/52	1	+	0.5 ir	h; t, m, dm	..	..	..	..
3068	*Agave roezliane*	Miahuatlan, Oax., Mex.	9/52	1	+	0.9 mi	.....	..	..	..	..
3258	*Agave roseana*	N. of Punta Frailes, B. C., Mex.	11/52	1	+	1.4 ir	t; h	..	..	..	..
3263	*Agave roseana*	N. of Punta Frailes, B. C., Mex.	11/52	1	+	1.7 ir	t; h	..	..	..	..
3279	*Agave roseana*	N. of Punta Frailes, B. C., Mex.	11/52	1	+	1.9 ir	h; t, g, m	..	..	..	..
3297	*Agave roseana*	N. of Punta Frailes, B. C., Mex.	11/52	1 dead	+	1.3 ir	t; g, h, dh	..	..	..	..
3307	*Agave roseana*	N. of Punta Frailes, B. C., Mex.	11/52	1	+	0	.....	..	..	..	..
3222	*Agave salmiana*	Cheran, Michoa, Mex.	10/52	1	+	0	.....	..	..	..	..
3184	*Agave scabra*	Chisos Mts., Tex.	10/52	fr	+	0.8 mi	m, dm; h	..	..	..	..
3338	*Agave scabra*	Big Bend Nat. Park, Alpine, Tex.	1/53	1	+	0	.....	..	..	..	..
3339	*Agave scabra*	Big Bend Nat. Park, Alpine, Tex.	1/53	fr, sd	+	0	.....	..	..	..	..
3340	*Agave scabra*	Big Bend Nat. Park, Alpine, Tex.	1/53	s	+	0	.....	..	..	..	..
3467	*Agave aff. schidigera*	Los Mochis, Sin., Mex.	3/53	1	+	1.2 ir	d; h, g, m	..	..	..	..
3463	*Agave schottii*	Bachaca, Son., Mex.	3/53	1	+	0.7 ir	c; t	..	..	..	..
3157	*Agave seemanniana*	Mesa de Copoya, Chia., Mex.	9/52	1	+	0	.....	..	..	..	..
3426	*Agave seemanniana*	Tegucigalpa, Hond.	3/53	1	+	0	.....	..	..	..	..
3251	*Agave sobria*	Bahia Concepcion, B. C., Mex.	12/52	1	+	0.2	h100	..	..	..	..
3268	*Agave sobria*	Comondu, B. C., Mex.	12/52	1	+	0.5 ir	h; t, m	..	..	..	..
3278	*Agave sobria*	Bahia Concepcion, B. C., Mex.	12/52	1	+	0	.....	..	..	..	..

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis Test	Sapogenins: Total, M. F. B., %	Identified, % of Total	Flavonoids	Alkaloids	Tannins	Sterols
				AMARYLLIDACEAE							
3290	*Agave sobria*	Comondu, B. C., Mex.	12/52	l dead	+	0.4 ir	m; g, h	..	..	..	..
3300	*Agave sobria*	Sierra Giganta, B. C., Mex.	12/52	l	+	0	.....	..	..	..	..
3301	*Agave sobria*	Bahia Concepcion, B. C., Mex.	12/52	l	+	0.2 ir	h; t, dh, m	..	..	..	..
3306	*Agave sobria*	Sierra Giganta, B. C., Mex.	12/52	l	+	0.6 ir	h; t, m	..	..	..	..
3267	*Agave sullivanii*	San Antonio, B. C., Mex.	11/52	l	+	1.3 ir	m, h; dmt	..	..	..	..
3457	*Agave toumeyana*	Magna Copper Mines, Ariz.	3/53	l	+	1.5 mi	.....	..	..	..	..
3492	*Agave toumeyana*	Sacaton, Ariz.	4/53	l	+	1.8 ir	h, m, dm, t; g	..	..	..	..
3555	*Agave toumeyana*	B. Benson, Kingman, Ariz.	11/53	l	+	1.5 ir	h, m, dm; g	..	..	..	..
3069	*Agave verschaffeltii*	N. E. of Cd. Oaxaca, Mex.	8/52	l	+	0.15	t65	..	..	..	..
3073	*Agave verschaffeltii*	Yanhuitlan, Oax., Mex.	9/52	l	+	0	.....	..	..	..	..
3075	*Agave verschaffeltii*	Miahuatlan, Oax., Mex.	9/52	l	+	0.6	t100	..	..	..	..
3082	*Agave verschaffeltii*	N. E. of Cd. Oaxaca, Mex.	9/52	fl	+	0	.....	..	..	..	..
3199	*Agave verschaffeltii*	Tehuacan, Pueb., Mex.	10/52	fr	+	0.9 ir	t; h	..	..	..	..
3151	*Agave aff. verschaffeltii*	Comitan, Chia., Mex.	9/52	l	+	0	.....	..	..	..	..
3271	*Agave vexans*	Comondu, B. C., Mex.	11/52	l dead	+	0.8 ir	h; m, dm	..	..	..	..
3292	*Agave vexans*	Comondu, Bl. C., Mex.	11/52	l	+	0.4 ir	h; dh, m	..	..	..	..
3314	*Agave vilmoriniana*	Hunt. Bot. Gard.	12/52	s	+	1.5 ir	sm; h	..	..	..	..
3317	*Agave vilmoriniana*	Hunt. Bot. Gard.	12/52	l dead	+	3.2	sm100	..	..	..	..
3462	*Agave vilmoriniana*	San Bernardo, San., Mex.	4/53	l	+	3.3	sm80; y15; g5	..	..	..	..
3070	*Agave sp.*	Ixtlan, Oax., Mex.	9/52	l	+	0	.....	..	..	..	..
3071	*Agave sp.*	Ixtlan, Oax., Mex.	9/52	l	+	0.05	h35; e40; m, dm25	..	..	..	..
3077	*Agave sp.*	N. of Miahuatlan, Oax., Mex.	9/52	l	+	0.4	t100	..	..	..	..
3102	*Agave sp.*	Tehuitzingo, Pueb., Mex.	9/52	l	+	0.8	ss100	..	..	..	..
3120	*Agave sp.*	N. of Devil's Riv., Del Rio, Tex.	10/52	l	+	1.0 mi	.....	..	..	..	..
3122	*Agave sp.*	San Mateo Cajones, Oax., Mex.	10/52	l	+	0.5 mi	.....	..	..	..	..
3123	*Agave sp.*	Tuxtla Gutierrez, Chia., Mex.	9/52	l	+	0.4	h100	..	..	..	..
3124	*Agave sp.*	Comitan, Chia., Mex.	9/52	l	+	0.15	h100	..	..	..	..
3126	*Agave sp.*	Sierra Zempoaltepec, Oax., Mex.	9/52	l	+	0.7	h; dh 80; ni20	..	..	..	..
3132	*Agave sp.*	Sierra Zempoaltepec, Oax., Mex.	9/52	l	+	0	.....	..	..	..	..
3138	*Agave sp.*	Cintalapa, Chia., Mex.	9/52	l	+	0.2 mi	.....	..	..	..	..
3141	*Agave sp.*	Sierra Zempoaltepec, Oax., Mex.	9/52	l	+	0.2 mi	.....	..	..	..	..
3148[a]	*Agave sp.*	Mitla, Oax., Mex.	10/52	Pulp	+	0	.....	..	..	..	..
3164	*Agave sp.*	Comitan, Chia., Mex.	9/52	l	+	0.2 mi	.....	..	..	..	..
3166	*Agave sp.*	San Mateo Cajones, Oax., Mex.	9/52	l	+	0	.....	..	..	..	..
3167	*Agave sp.*	Tehuantepec, Oax., Mex.	9/52	l	+	0.2 mi	.....	..	..	..	..
3190	*Agave sp.*	Jacala, Hid., Mex.	10/52	l	+	0	.....	..	..	..	..
3191	*Agave sp.*	Jacala, Hid., Mex.	10/52	l	+	0.4 ir	t	..	..	..	..
3220	*Agave sp.*	E. of Carapan, Michoa., Mex.	10/52	l	+	0	.....	..	..	..	..

3221	*Agave sp.*	Uruapan, Michoa., Mex.	10/52	l	+	0	.....	..	.	..	..	
3240	*Agave sp.*	Oax., Mex. (Boone Hallberg)	11/52	l	+	0.7 mi	.....	..	..	..	..	
3241	*Agave sp.*	Oax., Mex. (Boone Hallberg)	11/52	l	+	0	.....	..	..	..	..	
3242	*Agave sp.*	Oax., Mex. (Boone Hallberg)	11/52	l	−	...	.....	..	..	..	..	
3243	*Agave sp.*	Oax., Mex. (Boone Hallberg)	12/52	l	−	...	.....	..	..	..	..	
3255	*Agave sp.*	W. of San Jose d. Cabo, B. C., Mex.	11/52	l	+	1.5	h100	..	..	..	..	
3264	*Agave sp.*	N. of San Xavier, B. C., Mex.	12/52	l	+	0.2 ir	m; t, h, dm	..	..	..	..	
3315	*Agave sp.*	Gates Cactus Farm, Corona, Cal.	12/52	fr	+	0	.....	..	..	..	..	
3316	*Agave sp.*	Hunt. Bot. Gard.	12/52	l	+	0	.....	..	..	..	..	
3318	*Agave sp.*	Hunt. Bot. Gard.	12/52	Peduncle	+	0	.....	..	..	..	..	
3319	*Agave sp.*	Hunt. Bot. Gard.	12/52	l, fr	+	0	.....	..	..	..	..	
3346	*Agave sp.*	San Arate, el Progreso, Guat.	1/53	l	+	0	.....	..	..	..	..	
3349	*Agave sp.*	Chiquimula, Guat.	1/53	l	+	0	.....	..	..	..	..	
3350	*Agave sp.*	Rancho, el Progreso, Guat.	1/53	l	+	0	.....	..	..	..	..	
3352	*Agave sp.*	Dept. de Guatemala, Guat.	1/53	l	+	0	.....	..	..	..	..	
3387	*Agave sp.*	Chapultepec, Guat.	2/53	l	+	0	.....	..	..	..	..	
3424	*Agave sp.*	Perspire, Choluteca, Hond.	3/53	l	+	0	.....	..	..	..	..	
3425	*Agave sp.*	Perspire, Choluteca, Hond.	3/53	l	+	0	.....	..	..	..	..	
3427	*Agave sp.*	Puerto del Diablo, El Salv.	3/53	l	+	0.5 ir	t; g	..	..	..	..	
3466	*Agave sp.*	Los Mochis, Sin., Mex.	3/53	l	+	0.3 mi	.....	..	..	..	..	
3479	*Agave sp.*	San Vicente, Pinar del Rio, Cuba	4/53	l	−	...	.....	..	..	..	..	
3488	*Agave sp.*	Encinal, Ver., Mex.	4/53	l	+	0.2 ir	t, m, dm	..	..	..	..	
3495	*Agave sp.*	Djakarta, Indonesia	5/53	l (juice)	+	0	.....	..	..	..	..	
3496	*Agave sp.*	Djakarta, Indonesia	5/53	l (juice)	+	0	.....	..	..	..	..	
3499	*Agave sp.*	Djakarta, Indonesia	5/53	l (juice)	+	1.9 mi	.....	..	..	..	..	
3508	*Agave sp.*	Cadereyta, Quer., Mex.	5/53	l	+	0.1 ir	h; t, g	..	..	..	..	
3512	*Agave sp.*	Frenchman's Bay, St. Thomas Virgin Islands	5/53	l	+	0.4 ir	t; m, g	..	..	..	..	
3526	*Agave sp.*	La Cruz, Santa Clara, Cuba	6/53	l	+	1.4 ir	g; t	..	..	..	..	
3572	*Amaryllis bicolor*	Escuadron, Concepcion, Chile	12/55	bu	−	...	.....	0	0	0	+	
3437	*Furcraea cabuya*	San Andres, El Salv.	3/53	l	+	0.4 ir	t; g, m	0	0	0	0	
3100	*Furcraea sp.*	Tehuitzingo, Pueb., Mex.	9/52	l	+	0.2	t60; ni40	0	0	0	0	
3152	*Furcraea guatamalensis*	Zacatepec, Mixes, Oax., Mex.	9/52	l	+	0.2 ir	t	0	0	0	+	
3161	*Furcraea guatamalensis*	Chia., Mex.	10/52	l	+	0.2 mi	nss	0	0	0	+	
3351	*Furcraea sp.*	Dept. el Progreso, Guat.	1/53	l	+	1.3 ir	t; h, m	0	0	0	0	
3419	*Furcraea sp.*	E. of Choluteca, Hond.	2/53	l	+	0.5 ir	t; g, h, m	0	0	0	+	
3398	*Furcraea sp.*	Zamorano, Hond.	2/53	l	+	0.3 ir	h; t, g	0	0	0	0	
3435	*Furcraea sp.*	San Andres, El Salv.	3/53	l	+	1.1 ir	t; g, m, h	0	0	0	0	
3827	*Furcraea sp.*	Huanuco, Peru	4/54	l	+	0.4 mi	.....	0	0	0	+	
3828	*Furcraea sp.*	Huanuco, Peru	4/54	l	+	0.5 ir	t; h	0	0	0	0	
3829	*Furcraea sp.*	Huanuco, Peru	4/54	l	+	0.3 ir	t; h	0	0	0	+	
3545	*Hippeastrum sp.*	Chile	11/53	t	+	0	.....	0	0	0	+	
3098	*Manfreda brachystachys*	Sierra Ixtacihuatl, Mex.	9/52	fr	+	1.2	t30; g10; m30; c30	0	0	0	0	
3137	*Manfreda brachystachys*	Tuxtla Gutierrez, Chia., Mex.	10/52	w	+	0	.....	0	0	0	0	
3219	*Manfreda brachystachys*	Michoa., Mex.	10/52	w	+	0	.....	0	0	0	0	
3507	*Manfreda brachystachys*	Sierra Ixtacihuatl, Mex.	5/53	r	+	1.8 ir	g; t	0	0	0	0	

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				AMARYLLIDACEAE							
3072	*Manfreda maculata*	N. E. of Cd. Oaxaca-Ixtlan, Mex.	8/52	l	+	0.2	g50; h, dh20; d30	0	0	0	0
3101	*Manfreda maculta*	Sierra Ixtacihuatl, Mex.	9/52	l	+	1.5	t25; g35; c40	0	0	0	0
3080	*Manfreda pringlei*	N. E. of Cd. Oaxaca-Ixtlan, Mex.	9/52	l	+	0.7 mi	g100	0	0	0	0
3020	*Manfreda sp.*	N. of Naranjo-Taxco, Guer., Mex.	8/52	r	+	0.2	g75; d25	0	0	0	0
3023	*Manfreda sp.*	N. of Naranjo-Taxco, Guer., Mex.	8/52	r	+	0.3 mi	.....	0	0	0	0
3051	*Manfreda sp.*	Tepoztlan, Morel, Mex.	9/52	w	+	0.6	.....	0	0	0	
3103	*Manfreda sp.*	Sierra Ixtacihuatl, Mex.	9/52	r	+	0.1	g100	0	0	0	
3232	*Pancratium maritimum*	Karacali, Mugla, Turkey	11/52	bu	+	0	.....	0	+++	0	
				ANACARDIACEAE							
3016	*Cyrtocarpa procera*	W. of Iguala, Guer., Mex.	8/52	r	+	0	....	0	0	0	0
3056	*Schinopsis lorentzii*	C. G. F.	9/52	l	−	...	.....	++	0	0	++
3902	*Schinus molle*	Quilca, Lima, Peru	4/54	l, tw, b	−	...	.....	++	0	0	0
				ANNONACEAE							
3030	*Annona bullata*	C. G. F.	9/52	l	−	...	.....	0	0	0	0
				APOCYNACEAE							
3045	*Thexetia sp.*	Ixcateopan, Guer., Mex.	9/52	fr	+	0	.....	0	0	0	+
3601	*Urechites lutea*	Atkins Gard., Soledad, Cuba	2/54	l, s, fr	−	...	.....	0	+++	+	++
				AQUIFOLIACEAE							
3725	*Ilex coriacea*	Southport, Brunswick Co., N. C.	4/54	l, tw	−	...	.....	+	0	0	+
3589	*Ilex glabra*	Southport, Brunswick Co., N. C.	2/54	l, s	−	...	.....	++	0	0	+
3711	*Ilex glabra*	Wayne Co., Ga.	3/54	l	−	...	.....	0	0	+++	+
3715	*Ilex myrtifolia*	Wayne Co., Ga.	3/54	l	−	...	.....	+	0	0	+++
3680	*Ilex opaca*	Raleigh, N. C.	3/54	l, tw	−	...	.....	+	0	0	++
3778	*Ilex vomitoria*	McIntosh Co., Ga.	4/54	l, s	−	...	.....	0	0	0	++
				ARACEAE							
3874	*Dieffenbachia cordata*	Cayumba, Tingo Maria, Peru	4/54	l, s	−	...	.. ..	0	0	0	++
				ARALIACEAE							
3724	*Hedera helix*	Wake Co., N. C.	4/54	l, tw	+	0	.....	+	0	0	++
				ARISTOLOCHIACEAE							
3107	*Aristolochia sp.*	G. D. M.	9/52	l, s, r	−	...	.....	0	0	0	+
3648	*Hexastylis arifolia*	Oglethorpe Co., Ga.	3/54	l, s	−	...	.....	++	0	0	++
				BERBERIDACEAE							
3831	*Podophyllum peltatum*	Raleigh, N. C.	4/54	l	−	...	.....	+	0	0	+++

			BETULACEAE							
3929	*Alnus serrulata*	Oglethorpe Co., Ga.	4/54 l, s	−	...	.....	++	0	+++	+
			BIGNONIACEAE							
3987	*Amphilophium aschersonii*	Peru	4/54 l, s	−	...	.....	0	0	0	+
3534	*Argylia sp.*	Cuesta de los Cardas, Chile	11/53 t	+	0	.....	0	0	0	0
3857	*Callichlamys latifolia*	E. of Tingo Maria, Peru	4/54 l, s, r	−	...	.....	+	0	+	+
			BOMBACACEAE							
3031	*Pachira insignis*	C. G. F.	9/52 l	−	...	.....	0	0	0	0
			BORAGINACEAE							
3011	*Cordia nitida*	C. G. F.	8/52 l	−	...	.....	0	0	0	0
3034	*Cordia serratifolia*	C. G. F.	9/52 l	−	...	.....	0	0	0	+
3035	*Cordia serratifolia*	C. G. F.	9/52 l	−	...	.....	0	0	0	0
3945	*Cordia sp.*	W. of Tingo Maria, Peru	4/54 l, s	−	...	.....	0	0	0	+++
3903	*Cordia sp.*	Quilca, Lima, Peru	4/54 l, s	−	...	.....	0	0	0	+++
3801	*Heliotropium anchusaefolium*	Toombs Co., Ga.	4/54 l, s	−	...	.....	0	0	0	++
3878	*Heliotropium sp.*	Carmen Alto, Tingo Maria, Peru	4/54 l, s	−	...	.....	0	0	0	++
3990	*Heliotropium sp.*	Rd. to Puerto Nuevo, Tingo Maria, Peru	4/54 l, s	−	...	.....	0	0	0	+++
3775	*Lithospermum arvense*	Clarke Co., Ga.	4/54 l, s	+	0	.....	+	0	0	+
			BUXACEAE							
3342	*Simmondsia chinensis*	Aquanga, Cal.	12/52 l	−	...	.....	0	0	0	+
3343	*Simmondsia chinensis*	Aguanga, Cal.	12/52 l	−	...	.....	0	0	0	+
			CACTACEAE							
3324	*Ferocactus sp.*	Mexico City, Mex	12/52 l	+	0	.....	0	0	0	0
3277	*Peniocereus greggii*	N. of San Xavier, B. C., Mex.	12/52 r	+	0	.....	0	0	0	0
			CAMPANULACEAE							
3961	*Siphocampylus sp.*	Peru	4/54 l, s, b	−	...	.....	0	0	0	0
3899	*Lobelia decurrens*	Bet. Chancay and Huacho, Lima, Peru	4/54 l, s	−	...	.....	0	0	0	+
			CAPRIFOLIACEAE							
3937	*Lonicera maackii*	Clarke Co., Ga.	4/54 l, s	−	...	.....	+	0	0	+++
3691	*Sambucus canadensis*	Raleigh, N. C.	3/54 l, tw	−	...	.....	+	0	0	++
3795	*Sambucus simpsonii*	McIntosh Co., Ga.	4/54 l	−	...	.....	0	0	0	++
3840	*Viburnum nudum*	Wake Co., N. C.	4/54 l, tw, fl	−	...	.....	+	0	0	++
3837	*Viburnum prunifollum*	Raleigh, N. C.	4/54 l, fl	−	...	.....	+	0	0	++
3914	*Viburnum recognitum*	Southport, Brunswick Co., N. C.	4/54 l, tw	−	...	.....	+	0	0	+++
			CARYOPHYLLACEAE							
3647	*Stellaria media*	Clarke Co., Ga.	3/54 w	−	...	.....	0	0	0	++
			CASUARINACEAE							
3597	*Casuarina fraseri (cult.)*	Soledad, Cuba	2/54 l, s	−	...	.....	+	0	0	++
3007	*Casuarina montana*	C. G. F.	8/52 l	−	...	.....	0	0	0	+

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				CHENOPODIACEAE							
3769	*Chenopodium album*	Oglethorpe Co., Ga.	4/54	l, s	+	0	.....	0	0	0	+++
3782	*Salicornia perennis*	McIntosh Co., Ga.	4/54	l, s	+	0	.....	0	0	0	+
				CLAUSIACEAE							
3599	*Clusia rosea (cult.)*	Soledad, Cuba	2/54	l	−	...	.....	0	0	0	++
3870A	*Clusia sp.*	Tingo Maria Peru	4/54	l, s	−	...	.....	0	0	+	+
3870B	*Clusia sp.*	Tingo Maria, Peru	4/54	b	−	...	.....	0	0	+++	0
				COMBRETACEAE							
3008	*Terminalia calamansanay*	C. G. F.	8/52	l	−	...	.....	+	0	0	+
3054	*Terminalia superba*	C. G. F.	9/52	l	−	...	.....	+	0	+++	+
3004	*Terminalia triflora*	C. G. F.	8/52	l	−	...	.....	0	0	++	+
				COMMELIANCEAE							
3875	*Campelia zanonia*	Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	++
3856	*Dichlorisandra hexandra*	Tingo Maria, Peru	4/54	w	−	...	.....	0	0	0	++
3956	*Dichlorisandra hexandra*	Rio Monzon, Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	+++
3021	*Tradescantia sp.*	Iguala, Guer., Mex.	8/52	w	+	0	.....	0	0	0	0
				COMPOSITAE							
3791	*Baccharis angustifolia*	McIntosh Co., Ga.	4/54	l, s	−	...	.....	0	0	0	++
3726	*Baccharis halimifolia*	Southport, Brunswick Co., N. C.	4/54	l, tw	−	...	.....	+	0	0	++
3792	*Borrichia frutescens*	McIntosh Co., Ga.	4/54	l	+	0	.....	0	0	0	+
3683	*Centaurea cyanus*	Raleigh, N. C.	3/54	w	−	...	.....	0	0	0	+++
3805	*Centaurea cyanus*	Clarke Co., Ga.	4/54	l	−	...	.....	0	0	0	+++
3926	*Chrysanthemum leucanthemum*	Clarke Co., Ga.	4/54	l, s	−	...	.....	0	0	0	+++
3913	*Chrysogonum virginicum*	Southport, Brunswick Co., N. C.	4/54	l, fl	+	0	.....	0	0	0	++
3721	*Cichorium intybus*	Raleigh, N. C.	4/54	w	−	...	.....	0	0	0	++
3788	*Cirsium horridulum*	McIntosh Co., Ga.	4/54	l, s	−	...	.....	++	0	0	+
3974	*Clibadium divaricatum*	Bet. Puente Prado and Las Palmas, Tingo Maria, Peru	4/54	l, s	−	...	.....	++	0	0	++
3644	*Cnicus benedictus*	Clarke Co., Ga.	3/54	l	−	...	.....	+	0	0	++
3922	*Coreopsis basalis*	Southport, Brunswick Co., N. C.	4/54	w	−	...	.....	0	0	0	+
3841	*Erigeron annuus*	Raleigh, N. C.	4/54	l, tw	−	...	.....	0	0	0	+
3454	*Eupatorium sp.*	Cuyotenango, Dept. Such., Guat.	3/53	r	+	0	.....	0	0	0	+
3730	*Gaillardia pulchella*	Southport, Brunswick Co.	4/54	l	−	...	.....	0	0	0	++
3765	*Gnaphalium purpureum*	Oglethorpe Co., Ga.	4/54	l, s	+	0	.....	0	0	0	+
3904	*Helianthus sp.*	Quilca, Lima, Peru	4/54	l, s	+	0	.....	0	0	0	+
3649	*Heterotheca subaxillaris*	Oglethorpe Co., Ga.	3/54	l, s	−	...	.....	+	0	0	++
3727	*Iva frutescens*	Southport, Brunswick Co., N. C.	3/54	l, tw	−	...	.....	0	0	0	++

			COMPOSITAE								
3834	*Lactuca scariola*	Raleigh, N. C.	4/54 l	−	...	.....	0	0	0	++	
3891	*Liabum sagittatum*	Bet. Divisoria and Carmen Alto, Tingo Maria, Peru	4/54 l, s	−	...	.....	0	0	0	+	
3984	*Mikania micrantha*	E. of Tingo Maria, Peru	4/54 l, s	−	...	.....	+	0	0	+++	
3790	*Pyrrhopappus carolinianus*	Sapelo Isl., Ga.	4/54 l	−	...	.....	0	0	0	+	
3972	*Schistocarpa oppositifolia*	Bet. Puente Prado and Las Palmas, Tingo Maria, Peru	4/54 l, s	−	...	.....	0	0	0	++	
3690	*Senecio smallii*	Raleigh, N. C.	3/54 l	−	...	.....	+	0	0	++	
3705	*Senecio vulgaris*	Oglethorpe Co., Ga.	3/54 l	−	...	.....	0	0	0	++	
3709	*Sonchus asper*	Wayne Co., Ga.	3/54 l	−	...	.....	+	0	0	+	
3722	*Taraxacum officinale*	Raleigh, N. C.	4/54 w	−	...	.....	0	0	0	++	
3994A	*Tessaria integrifolia*	Rd. to Puerto Nuevo, Tingo Maria, Peru	4/54 l, s	−	...	.....	++	0	0	+	
3994B	*Tessaria integrifolia*	Rd. to Puerto Nuevo, Tingo Maria, Peru	4/54 b	−	...	.....	0	0	0	0	
3907	*Trixis sp.*	Quilca, Lima, Peru	4/54 l, s	−	...	.....	0	0	0	+	
3847	*Wulffia baccata*	Lower Rio Monzon, Tingo Maria, Peru	4/54 l, tw	+	0	.....	0	0	0	+	
			CONVOLVULACEAE								
3333	*Ipomoea asarifolia*	G. D. M.	1/53 r, s, l	−	...	.....	+	0	0	0	
3327	*Ipomoea murucoides*	Mexico City, Mex.	12/52 l	−	...	.....	..	..	..	..	
3108	*Ipomoea sp.*	G. D. M.	9/52 l, s	−	...	.....	0	0	0	+	
3022	*Ipomoea sp.*	Naranjo, Guer., Mex.	8/52 r	+	0	.....	0	0	0	0	
			CRUCIFERAE								
3833	*Arabidopsis thaliana*	Raleigh, N. C.	4/54 w	−	...	.....	++	0	0	++	
3768	*Arabidopsis thaliana*	Oglethorpe Co., Ga.	4/54 l, s	−	...	.....	+	0	0	+++	
3278	*Cakile edentula*	Southport, Brunswick Co., N. C.	4/54 w	−	...	.....	+	0	0	+	
3781	*Cakile harperi*	McIntosh Co., Ga.	4/54 l, s	−	...	.....	0	0	0	+	
3646	*Capsella bursa-pastoris*	Clarke Co., Ga.	3/54 w	−	...	.....	0	0	0	++	
3676	*Capsella bursa-pastoris*	Raleigh, N. C.	3/54 w	−	...	.....	+	0	0	++	
3763	*Coronopus didymus*	Oglethorpe Co., Ga.	4/54 l, s	−	...	.....	0	0	0	++	
3923	*Descurainia pinnata*	Southport, Brunswick Co., N. C.	4/54 w	−	...	.....	0	0	0	+	
3766	*Sibara virginica*	Oglethorpe Co., Ga.	4/54 l, s	−	...	.....	+	0	0	+	
			CYCLANTHACEAE								
3657	*Carludovica sp.*	C. G. F.	3/54 l, s	−	...	.....	0	0	0	++	
			CYPERACEAE								
3989	*Cyperus esculentus*	E. of Tingo Maria, Peru	4/54 l, s, r	−	...	.....	0	0	0	+	
			CYRILLACEAE								
3762	*Cliftonia monophylla*	Wayne Co., Ga.	4/54 l, s	−	...	.....	++	0	+++	+	
			DIOSCOREACEAE								
3165	*Dioscorea aguilarii*	Tuxtla, Gutierrez, Chia., Mex.	10/52 r	+	0	.....	..	..	..	..	
3573	*Dioscorea auriculata*	Rio de las Cruces, Chile	12/53 t	+	0.2 mi ni		..	..	..	..	

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis Test	Sapogenins: Total, M. F. B., %	Sapogenins: Identified, % of Total	Flavonoids	Alkaloids	Tannins	Sterols
				DIOSCOREACEAE							
3125	*Dioscorea bartlettii*	Rancho Ocote, Chia., Mex.	10/52	r	+	0.33	ya100	..	..	..	..
3385	*Dioscorea bartlettii*	Sebol, Alta Verapaz, Guat.	2/53	r, t	+	0	.....	..	..	..	..
3389	*Dioscorea bartlettii*	Panzos, Alta Verapaz, Guat.	2/53	t	+	0.8	d50, ya50	..	..	..	..
3485	*Dioscorea bartlettii*	El Palmar, Ver., Mex.	5/53	r	+	0.3	ya80:d20	..	..	..	..
3113	*Dioscorea composita*	G. D. M.	9/52	rh	+	0.2 mi	d100	..	..	..	..
3200[b]	*Dioscorea composita*	Tierra Blanca, Ver., Mex.	10/52	r	+	10.1[a]	d100	..	..	..	..
3201	*Dioscorea composita*	Tuxtepec, Oax., Mex.	10/52	r	+	3.1	d100	..	..	..	..
3202	*Dioscorea composita*	Tuxtepec, Oax., Mex.	10/52	r	+	2.4	d100	..	..	..	..
3384	*Dioscorea composita*	Sebol, Alta Verapaz, Guat.	2/53	r	+	0.03	d100	..	..	..	..
3390	*Dioscorea composita*	Panzos, Alta Verapaz, Guat.	2/53	r	+	0	.....	..	..	..	..
3392	*Dioscorea composita*	W. of Estor, Izabal, Guat.	2/53	r	+	2.6	d90; ni10	..	..	..	..
3450	*Dioscorea composita*	Sinahu, Alta Verapaz, Guat.	3/53	t	+	0	.....	..	..	..	..
3486	*Dioscorea composita*	El Palmar, Ver., Mex.	5/53	t	+	5.0	d90; ya10	..	..	..	..
3487	*Dioscorea composita*	Sierra Blanca, Ver., Mex.	5/53	t	+	0	.....	..	..	..	..
3501	*Dioscorea composita*	Martinez de la Torre, Ver., Mex.	5/53	t	+	2.9	d100	..	..	..	..
3504	*Dioscorea composita*	El Suchill, Ver., Mex.	5/53	t	+	5.4	d100	..	..	..	..
3505	*Dioscorea composita*	Huauchinango, Ver., Mex.	5/53	t	+	3.4	d100	..	..	..	..
3518	*Dioscorea composita*	G. D. M.	4/53	r, t	+	0	.....	..	..	..	..
3519	*Dioscorea composita*	G. D. M.	4/53	r, t	+	0	.....	..	..	..	..
3048	*Dioscorea convolvulacea*	Cuernavaca, Morel., Mex.	9/52	rh	+	0	.....	..	..	..	..
3461	*Dioscorea cotinifolia*	Natal, S. Africa	2/53	r	+	0	.....	0	0	0	0
3442	*Dioscorea dregeana*	Natal, S. Africa	2/53	r	+	0	.....	..	..	..	..
3135	*Dioscorea dugesii*	Turtla Gutierrez, Chia., Mex.	10/52	r	+	0	.....	..	..	..	..
3171	*Dioscorea hemicrypta*	G. D. M.	10/52	t	+	0	.....	..	..	..	..
3015	*Dioscorea lobata*	W. of Iguala, Guer., Mex.	8/52	rh	+	0	.....	+	0	0	+
3136	*Dioscorea macrostachya*	Rancho Ocote, Chia., Mex.	10/52	r	+	0.3	d100	..	..	..	..
3347	*Dioscorea macrostachya*	El, Progreso, Guat.	1/53	t	+	0.15	d + ya100	..	..	..	..
3402	*Dioscorea macrostachya*	S. W. of La Ceiba, Atlantida, Hond.	3/53	r	+	1.5	d100	..	..	..	..
3353	*Dioscorea nelsonii*	La Lima, Hond.	1/53	r	+	0.04	d100	..	..	..	..
3403	*Dioscorea nelsonii*	Lancetilla Sta., Atlantida, Hond.	3/53	r	+	1.4	d100	..	..	..	..
3404	*Dioscorea nelsonii*	W. of Ceiba, Atlantida, Hond.	3/53	r	+	1.8	d100	..	..	..	..
3018	*Dioscorea platycolpota*	W. of Iguala, Guer., Mex.	8/52	rh	+	0.4	d100	+	0	0	0
3397	*Dioscorea polygonoides*	Zamorano, Hond.	2/53	r	+	0.25	d100	..	..	..	..
3570	*Dioscorea saxatilis*	Rancagua, O'Higgins Prov., Chile	11/53	r	+	0	.....	..	..	..	..
3571	*Dioscorea saxatilis*	Rancagua, O'Higgins Prov., Chile	11/53	r	+	0	.....	..	..	..	..
3178	*Dioscorea sinuata*	G. D. M.	10/52	t	+	0	.....	..	..	..	..
3140	*Dioscorea spiculiflora*	Tuxtla Gutierrez, Chia., Mex.	10/52	r	+	1.5	d+ya75; co+ge25	..	..	..	..
3513	*Dioscorea spiculiflora*	Tuxtla Gutierrez, Chia., Mex.	6/53	t	+	2.7	d+ya85; co+ge15	..	..	..	..
3823	*Dioscorea stegelmanniana*	Huallaga Riv., Tingo Maria, Peru	4/54	rh	+	0	.....	..	..	..	..

DIOSCOREACEAE

3044	*Dioscorea subtomentosa*	Cuernavaca, Morel., Mex.	9/52	rh	+	0	.....	..	..	..	..	
3443	*Dioscorea sylvatica*	Natal, S. Africa	3/53	r	+	2.4 mi	d100	..	..	..	..	
3110	*Dioscorea tepinapensis*	G. D. M.	9/52	rh	+	0.5 mi	d100	..	..	..	..	
3554	*Dioscorea tepinapensis*	Brooksville, Fla.	11/53	r	+	0.4 mi	d100	..	..	..	..	
3556	*Dioscorea tepinapensis*	Baton Rouge, La.	11/53	r	+	0.7 mi	d100	..	..	..	..	
3522	*Dioscorea urophylla*	Pedro Miguel, Canal Zone	6/53	t	+	0	.....	..	..	..	..	
3523	*Dioscorea urophylla*	Pedro Miguel, Canal Zone	6/53	r	+	0	.....	..	..	..	..	
3524	*Dioscorea urophylla*	Pedro Miguel, Canal Zone	6/53	t	+	0	.....	..	..	..	..	
3580	*Dioscorea valdiviensis*	Cautin, Chile	1/54	t	+	0	.....	..	..	..	..	
3001	*Dioscorea sp.*	N. of Tihuatlan, Ver., Mex.	8/52	l	+	2.3	d95; p5	..	..	..	..	
3014	*Dioscorea sp.*	W. of Iguala, Guer., Mex.	8/52	rh	+	0	.....	..	..	..	..	
3025	*Dioscorea sp.*	Tegucigalpa, Hond.	8/52	rh	+	0.4 mi	d100	..	..	..	..	
3046	*Dioscorea sp.*	Cuernavaca, Morel., Mex.	9/52	t	+	0	.....	..	..	..	..	
3050	*Dioscorea sp.*	Cuernavaca, Morel., Mex.	9/52	rh	+	0	.....	..	..	..	..	
3052	*Dioscorea sp.*	Ixcateopan, Guer., Mex.	9/52	rh	+	0	.....	..	..	..	..	
3067	*Dioscorea sp.*	Ver., Mex. (J. W. Ayers)	9/52	rh	+	8.5	d100	..	..	..	..	
3111	*Dioscorea sp.*	G. D. M.	9/52	rh	+	0.5 mi	d100	..	..	..	..	
3112	*Dioscorea sp.*	G. D. M.	9/52	rh	+	0.2 mi	d100	..	..	..	..	
3114	*Dioscorea sp.*	G. D. M.	9/52	rh	+	1.6 mi	d100	..	..	..	..	
3115	*Dioscorea sp.*	G. D. M.	9/52	rh	+	0.5 mi	d100	..	..	..	..	
3116	*Dioscorea sp.*	Mexico City, D. F., Mex.	9/52	rh	+	2.7	d100	..	..	..	..	
3127	*Dioscorea sp.*	Comitan, Chia., Mex.	10/52	r	+	4.2	d50; ya50	..	..	..	..	
3129	*Dioscorea sp.*	Tuxtla Gutierrez, Chia., Mex.	10/52	r	+	0	.....	..	..	..	..	
3133	*Dioscorea sp.*	Tuxtla Gutierrez, Chia., Mex.	10/52	r	+	1.8	d100	..	..	..	..	
3134	*Dioscorea sp.*	Cerro Juarez, Chia., Mex.	10/52	r	+	2.4	d+ya75; ge+co25	..	..	..	..	
3143	*Dioscorea sp.*	Bochil, Chia., Mex.	10/52	r	+	0	.....	..	..	..	..	
3145	*Dioscorea sp.*	Rancho Ocote, Chia., Mex.	10/52	r	+	0.2	nss	..	..	..	..	
3146	*Dioscorea sp.*	Zanatepec, Oax., Mex.	10/54	r	+	0	.....	..	..	..	..	
3147	*Dioscorea sp.*	Sierra Zempoaltepec, Oax., Mex.	10/52	r	+	0	.....	..	..	..	..	
3150	*Dioscorea sp.*	Camaron, Oax., Mex.	10/52	r	+	0	.....	..	..	..	..	
3153	*Dioscorea sp.*	Ocotal, Chia., Mex.	10/52	r	+	0	.....	..	..	..	..	
3155	*Dioscorea sp.*	Comitan, Chia., Mex.	10/52	r	+	0	.....	..	..	..	..	
3159	*Dioscorea sp.*	Tuxtla Gutierrez, Chia., Mex.	10/52	r	+	0	.....	..	..	..	..	
3168	*Dioscorea sp.*	G. D. M.	10/52	t	+	1.6 mi	d100	..	..	..	..	
3169	*Dioscorea sp.*	G. D. M.	10/52	t	+	0	.....	..	..	..	..	
3170	*Dioscorea sp.*	G. D. M.	10/52	t	+	0	.....	..	..	..	..	
3172	*Dioscorea sp.*	G. D. M.	10/52	t	+	0	.....	..	..	..	..	
3173	*Dioscorea sp.*	G. D. M.	10/52	t	+	0	.....	..	..	..	..	
3174	*Dioscorea sp.*	G. D. M.	10/52	t	+	0	.....	..	..	..	..	
3175	*Dioscorea sp.*	G. D. M.	10/52	t	+	0	.....	..	..	..	..	
3176	*Dioscorea sp.*	G. D. M.	10/52	t	+	0	.....	..	..	..	..	
3177	*Dioscorea sp.*	G. D. M.	10/52	t	+	0	.....	..	..	..	..	
3179	*Dioscorea sp.*	G. D. M.	10/52	t	+	0	.....	..	..	..	..	
3180	*Dioscorea sp.*	G. D. M.	10/52	t	+	0	.....	..	..	..	..	
3181	*Dioscorea sp.*	G. D. M.	10/52	t	+	0	.....	..	..	..	..	
3182	*Dioscorea sp.*	G. D. M.	10/52	t	+	0	.....	..	..	..	..	

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis Test	Sapogenins: Total, M. F. B., %	Sapogenins: Identified, % of Total	Flavonoids	Alkaloids	Tannins	Sterols
				DIOSCOREACEAE							
3185	*Dioscorea sp.*	Puerto Barrios, Izabal, Guat.	10/52	tb	+	2.3	d99; p1	..	..	..	..
3186	*Dioscorea sp.*	Puerto Barrios, Izabal, Guat.	10/52	r	+	0.5	d100	0	0	0	+
3187	*Dioscorea sp.*	Puerto Barrios, Izabal, Guat.	10/52	r	+	2.1	d100	..	..	..	..
3188	*Dioscorea sp.*	Puerto Barrios, Izabal, Guat.	10/52	s, l	+	0	.....	0	0	+++	+
3198	*Dioscorea sp.*	Cordoba, Ver., Mex.	10/52	r	+	0	.....	..	..	..	..
3209	*Dioscorea sp.*	Puerto Barrios, Izabal, Guat.	10/52	r	−	...	.....	0	0	0	0
3210	*Dioscorea sp.*	Puerto Barrios, Izabal, Guat.	10/52	r	−	...	.....	+	0	0	0
3211	*Dioscorea sp.*	Puerto Barrios, Izabal, Guat.	10/52	r	+	0.8	d100	0	0	0	0
3212	*Dioscorea sp.*	Puerto Barrios, Izabal, Guat.	10/52	r	+	0.15	d75; ni25	+	0	0	0
3223	*Dioscorea sp.*	Apatzingan, Michoa., Mex.	10/52	t	+	4.4	d100	..	..	..	..
3226	*Dioscorea sp.*	Puerto Barrios, Izabal, Guat.	10/52	r	+	0	.....	+	0	+++	0
3227	*Dioscorea sp.*	Puerto Barrios, Izabal, Guat.	10/52	r	+	0.3	d100	0	0	+	0
3228	*Dioscorea sp.*	Puerto Barrios, Izabal, Guat.	10/52	s, l	+	0	.....	+	0	++	0
3229	*Dioscorea sp.*	Puerto Barrios, Izabal, Guat.	10/52	t	+	0	.....	0	0	0	0
3230	*Dioscorea sp.*	Puerto Barrios, Izabal, Guat.	10/52	r	+	1.7	d100	0	0	0	0
3231	*Dioscorea sp.*	Puerto Barrios, Izabal, Guat.	10/52	r	+	1.7 mi	d100	0	0	0	0
3234	*Dioscorea sp.*	Guatamala City, Guat.	10/52	r	+	0	.....	0	0	0	0
3235	*Dioscorea sp.*	Guatamala City, Guat.	10/52	r	+	0.5	d100	..	..	..	..
3236	*Dioscorea sp.*	Guatamala City, Guat.	10/52	b	−	...	.....	+	0	++	+
3237	*Dioscorea sp.*	Guatamala City, Guat.	10/52	s	−	...	.....	0	0	0	0
3238	*Dioscorea sp.*	Guatamala City, Guat.	10/52	r	−	...	.....	0	0	0	0
3239	*Dioscorea sp.*	Turrialba, Costa Rica	12/52	t	+	2.3	d+ya100	..	..	..	..
3348	*Dioscorea sp.*	Cruz, El Progreso, Guat.	1/53	t	+	0.3	d100	..	..	..	..
3354	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3355	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3356	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3357	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3358	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3359	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3360	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3361	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3362	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3363	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3364	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3365	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3366	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3367	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3368	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3369	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3370	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3371	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3372	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0.8 mi	d100	..	..	..	..
3373	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0.5 mi	d100	..	..	..	..
3374	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..

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3375	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3376	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3377	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3378	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3379	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3380	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3381	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3382	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3383	*Dioscorea sp.*	Mayaguez, P. R.	1/53	t	+	0	.....	..	..	..	..
3386	*Dioscorea sp.*	Sebol, Alta Verapaz, Guat.	2/53	r	+	0	.....	..	..	..	..
3388	*Dioscorea sp.*	Sebol, Alta Verapaz, Guat.	2/53	r	+	1.0	d100	..	..	..	..
3391	*Dioscorea sp.*	Panzos, Alta Verapaz, Guat.	2/53	r	+	1.0	d100	..	..	..	..
3393	*Dioscorea sp.*	Panzos, Alta Verapaz, Guat.	2/53	r	+	2.3	d100	..	..	..	..
3394	*Dioscorea sp.*	Panzos, Alta Verapaz, Guat.	2/53	r	+	0.3 mi	nss	..	..	..	..
3405	*Dioscorea sp.*	W. of San Pedro Sula, Hond	3/53	r	+	0	.....	..	..	..	..
3406	*Dioscorea sp.*	Cofradia, Cortes, Hond.	3/53	r	+	0	.....	..	..	..	..
3407	*Dioscorea sp.*	Lancetilla Sta., Atlantida, Hond.	3/53	r	+	0	.....	..	..	..	..
3408	*Dioscorea sp.*	Lancetilla Sta., Atlantida, Hond.	3/53	r	+	0	.....	..	..	..	..
3409	*Dioscorea sp.*	Lancetilla Sta., Atlantida, Hond.	3/53	r	+	0.3	d100	..	..	..	..
3410	*Dioscorea sp.*	Lancetilla Sta., Atlantida, Hond.	3/53	t	+	0	.....	..	..	..	..
3411	*Dioscorea sp.*	W. of La Ceiba, Atlantida, Hond.	3/53	r	+	0.5	d100	..	..	..	..
3412	*Dioscorea sp.*	S. of La Ceiba, Atlantida, Hond.	3/53	r	+	0.5	d100	..	..	..	..
3413	*Dioscorea sp.*	S. of La Ceiba, Atlantida, Hond.	3/53	r	+	0.2	d100	..	..	..	..
3414	*Dioscorea sp.*	S. W. of La Ceiba, Atlantida, Hond.	3/53	r	+	0.15	d100	..	..	..	..
3417	*Dioscorea sp.*	Lancetilla Sta., Atlantida, Hond.	3/53	t	+	0	.....	..	..	..	..
3418	*Dioscorea sp.*	E. of Choluteca, Hond.	3/53	r	+	0	.....	..	..	..	..
3420	*Dioscorea sp.*	Tablones, Choluteca, Hond.	3/53	r	+	1.0	d100	..	..	..	..
3421	*Dioscorea sp.*	Perspire, Choluteca, Hond.	3/53	r	+	3.2	d100	..	..	..	..
3422	*Dioscorea sp.*	Perspire, Choluteca, Hond.	3/53	r	+	0	.....	..	..	..	..
3423	*Dioscorea sp.*	Perspire, Choluteca, Hond.	3/53	r	+	0	.....	..	..	..	..
3428	*Dioscorea sp.*	Pta. Libertad, El Salv.	3/53	t	+	0	.....	..	..	..	..
3429	*Dioscorea sp.*	Pta. Libertad, El Salv.	3/53	t	+	0	.....	..	..	..	..
3430	*Dioscorea sp.*	Pta. Libertad, El Salv.	3/53	r	+	3.5	ya75; d25	..	..	..	..
3431	*Dioscorea sp.*	Sta. Cruz Porrillo, El Salv.	3/53	r	+	1.35	d+ya100	..	..	..	..
3432	*Dioscorea sp.*	Sta. Cruz Porrillo, El Salv.	3/53	r	+	0	.....	..	..	..	..
3433	*Dioscorea sp.*	Sta. Cruz Porrillo, El Salv.	3/53	r	+	0	.....	..	..	..	..
3434	*Dioscorea sp.*	S. of San Salvador, El Salv.	3/53	r	+	0	.....	..	..	..	..
3439	*Dioscorea sp.*	Pedro Miguel, Canal Zone	3/53	t	−	...	.....	..	..	..	..
3440	*Dioscorea sp.*	Pedro Miguel, Canal Zone	3/53	t	−	...	.....	..	..	..	..

Accession No.	Species	Collection Source	Date Collected	Plant Part	Hemolysis Test	Sapogenins Total, M. F. B., %	Sapogenins Identified, % of Total	Flavonoids	Alkaloids	Tannins	Sterols
				DISOCOREACEAE							
3441	*Dioscorea sp.*	Pedro Miguel, Canal Zone	3/53	r	+	0	.....	..	..	..	..
3446	*Dioscorea sp.*	G. D. M.	3/53	t	+	0	.....	..	..	..	..
3447	*Dioscorea sp. (cult.)*	Baton Rouge, La.	3/53	t	+	0	.....	..	..	..	..
3448	*Dioscorea sp. (cult.)*	Baton Rouge, La.	3/53	t	+	0	.....	..	..	..	..
3449	*Dioscorea sp. (cult.)*	Baton Rouge, La.	3/53	t	+	0	.....	..	..	..	..
3451	*Dioscorea sp. (cult.)*	Cuyotenango, Guat.	3/53	t	+	0	.....	..	..	..	..
3452	*Dioscorea sp. (cult.)*	Cuyotenango, Guat.	3/53	t	+	0	.....	..	..	..	..
3455	*Dioscorea sp.*	San Sebastian, Retal., Guat.	3/53	r	+	0.9	d45; ya45	..	..	..	..
3456	*Dioscorea sp.*	San Rafael Panan, Guat.	3/53	r	+	3.0	d75; ya25	..	..	..	..
3468	*Dioscorea sp.*	Tepic-Mazatlan, Nay., Mex.	4/53	r	+	3.7	d100	..	..	..	..
3469	*Dioscorea sp.*	W. of Jalcocotan, Nay., Mex.	4/53	r	+	2.5	d100	..	..	..	..
3481	*Dioscorea sp.*	Tuxtepec, Oax., Mex.	5/53	t	+	3.0	d100	..	..	..	..
3484	*Dioscorea sp.*	La Tinaja and Tierra Blanca, Ver., Mex.	5/53	r	+	2.4	d+ya100	..	..	..	..
3489	*Dioscorea sp.*	Jalapa, Ver., Mex.	5/53	r	+	1.7	ya80; d20	..	..	..	..
3503	*Dioscorea sp.*	Tihautlan, Ver., Mex.	5/53	r	+	3.8	d100	..	..	..	..
3510	*Dioscorea sp.*	Turrialba, C. R.	5/53	t	+	2.1	d100	..	..	..	..
3511	*Dioscorea sp.*	E. End St. Croix, Virgin Islands	6/53	t	+	0.3	ni	..	..	..	..
3520	*Dioscorea sp.*	G. D. M.	4/53	t	+	0	.....	..	..	..	..
3521	*Dioscorea sp.*	G. D. M.	4/53	t	+	0	.....	..	..	..	..
3525	*Dioscorea sp.*	Pedro Miguel, Canal Zone	6/53	t	+	0	.....	..	..	..	..
3532	*Dioscorea sp.*	Savannah, Ga.	9/53	t	+	0	.....	..	..	..	..
3535	*Dioscorea sp.*	Valparaiso, Chile	11/53	t	+	0.6 mi	d100	..	..	..	..
3536	*Dioscorea sp.*	Nat. Bot. Gard., Valparaiso, Chile	11/53	t	+	0	.....	..	..	..	..
3537	*Dioscorea sp.*	Nat. Bot. Gard., Valparaiso, Chile	11/53	t	+	0	.....	..	..	..	..
3538	*Dioscorea sp.*	Nat. Bot. Gard., Valparaiso, Chile	11/53	t	+	0	.....	..	..	..	..
3539	*Dioscorea sp.*	Nat. Bot. Gard., Valparaiso, Chile	11/53	t	+	0	.....	..	..	..	..
3540	*Dioscorea sp.*	Nat. Bot. Gard., Valparaiso, Chile	11/53	t	+	0	.....	..	..	..	..
3541	*Dioscorea sp.*	Nat. Bot. Gard., Valparaiso, Chile	11/53	t	+	0	.....	..	..	..	..
3542	*Dioscorea sp.*	S. of Ovalle, Coquimbo, Chile	11/53	t	+	0	.....	..	..	..	..
3543	*Dioscorea sp.*	S. of Ovalle, Coquimbo, Chile	11/53	t	+	0	.....	..	..	..	..
3544	*Dioscorea sp.*	Gutierrez, Coquimbo, Chile	11/53	t	+	0	.....	..	..	..	..
3547	*Dioscorea sp.*	Savannah, Ga.	10/53	l, s	+	0	.....	..	..	..	..
3549	*Dioscorea sp.*	Savannah, Ga.	10/53	l, s	−	0	.....	..	..	..	..
3550	*Dioscorea sp.*	Savannah, Ga.	10/53	l, s	+	0	.....	..	..	..	..
3551	*Dioscorea sp.*	Savannah, Ga.	10/53	l, s	+	0	.....	..	..	..	..
3552	*Dioscorea sp.*	Savannah, Ga.	10/53	l, s	−	...	.....	..	..	..	..
3553	*Dioscorea sp.*	Brooksville, Fla.	11/53	r	+	0.6 mi	d100	..	..	..	..
3564	*Dioscorea sp. (cult.)*	Beltsville, Md.	12/53	l, s	−	...	.....	..	..	..	..

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3565	*Dioscorea sp. (cult.)*	Beltsville, Md.	12/53	l, s	+	0	.....	..	..	..	..	
3566	*Dioscorea sp. (cult.)*	Beltsville, Md.	12/53	l	−	...	.....	..	..	..	..	
3567	*Dioscorea sp.*	Valparaiso Prov., Chile	11/53	r	+	0	.....	..	..	..	..	
3568	*Dioscorea sp.*	Chile	12/53	r	+	0	.....	..	..	..	..	
3575	*Dioscorea sp.*	Osorno Prov., Chile	1/54	t	+	1.0	d100	..	..	..	..	
3576	*Dioscorea sp.*	Pedro Miguel, Canal Zone	1/54	t	−	...	.....	..	..	..	..	
3577	*Dioscorea sp.*	Pedro Miguel, Canal Zone	1/54	r	0	...	.....	..	..	..	..	
3578	*Dioscorea sp.*	W. of Osorno, Chile	1/54	t	+	0	.....	..	..	..	..	
3579	*Dioscorea sp.*	Valdivia Prov., Chile	1/54	t	+	0	.....	..	..	..	..	
3581	*Dioscorea sp.*	Noble Prov., Chile	1/54	rh	+	0.8	d100	..	..	..	..	
3569	*Dioscorea sp.*	Santiago Prov., Chile	12/53	r	+	0	.....	..	..	..	..	
3584	*Dioscorea sp.*	India	2/54	r	+	0	.....	0	0	0	0	
3585	*Dioscorea sp.*	Balamani Estate, Coorg, India	2/54	r	+	0	.....	0	0	0	0	
3600	*Dioscorea sp.*	Pedro Miguel, Canal Zone	2/54	t	−	...	.....	..	..	..	..	
3616	*Dioscorea sp.*	India	3/54	r	−	...	.....	..	..	..	..	
3617	*Dioscorea sp.*	Chacquear, Bolivia	3/54	t	−	...	.....	0	0	0	0	
3618	*Dioscorea sp.*	Cochabamba, Bolivia	3/54	r, t	−	...	.....	0	0	0	0	
3629	*Dioscorea sp.*	La Paz, Bolivia	3/54	t	−	...	.....	..	..	..	..	
3630	*Dioscorea sp.*	E. of La Paz, Bolivia	3/54	t	−	...	.....	..	..	..	..	
3632	*Dioscorea sp.*	Chulumani, La Paz, Bolivia	3/54	t	−	...	.....	..	..	..	..	
3633	*Dioscorea sp.*	Chulumani, La Paz, Bolivia	3/54	t	+	1.9	d100	..	..	..	..	
3634	*Dioscorea sp.*	Chulumani, La Paz, Bolivia	3/54	t	−	...	.....	..	..	..	..	
3635	*Dioscorea sp.*	Chulumani, La Paz, Bolivia	3/54	t	−	...	.....	..	..	..	..	
3735	*Dioscorea sp.*	G. D. M.	4/54	t	+	0.4 mi	ni	..	..	..	..	
3736	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3737	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3738	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3739	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3740	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3741	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3742	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3743	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3744	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3745	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3746	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3747	*Dioscorea sp.*	G. D. M.	4/54	t	+	0.2 mi	ni	..	..	..	..	
3748	*Dioscorea sp.*	G. D. M.	4/54	t	+	0	.....	..	..	..	..	
3749	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3750	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3751	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3752	*Dioscorea sp.*	G. D. M.	4/54	t	+	0.2 mi	.....	..	..	..	..	
3753	*Dioscorea sp.*	G. D. M.	4/54	t	+	0.7 mi	d100	..	..	..	..	
3754	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3755	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..	
3756	*Dioscorea sp.*	G. D. M.	4/54	t	+	0	.....	..	..	..	..	
3757	*Dioscorea sp.*	G. D. M.	4/54	t	+	0.6 mi	d100	..	..	..	..	

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis Test	Sapogenins: Total, M. F. B., %	Sapogenins: Identified, % of Total	Flavonoids	Alkaloids	Tannins	Sterols
				DIOSIOREACEAE							
3758	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..
3759	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..
3760	*Dioscorea sp.*	G. D. M.	4/54	t	+	0.9 mi	d100	..	..	..	..
3761	*Dioscorea sp.*	G. D. M.	4/54	t	−	...	.....	..	..	..	..
3820	*Dioscorea sp.*	E. of Huanuco, Peru	4/54	rh	−	0	.....	..	..	..	..
3821	*Dioscorea sp.*	E. of Carpish Pass, Peru	4/54	rh	+	0	.....	..	..	..	..
3822	*Dioscorea sp.*	E. of Carpish Pass, Peru	4/54	rh	+	0	.....	..	..	..	..
3824	*Dioscorea sp.*	Tingo Maria, Peru	4/54	rh	+	0.5 mi	ya100	..	..	..	..
3825	*Dioscorea sp.*	Tingo Maria, Peru	4/54	rh	+	0	.....	..	..	..	..
3826	*Dioscorea sp.*	W. of Carmen Alto, Tingo Maria, Peru	4/54	rh	+	1.0 mi	ni	..	..	..	..
3980	*Dioscorea sp.*	Tingo Maria, Peru	4/54	l, s	+	0	.....	0	0	0	+
				ELAEAGNACEAE							
3806	*Elaeagnus umbellatus*	Clarke Co., Ga.	4/54	l, tw	−	...	.....	0	0	0	+
				ERICACEAE							
3557	*Arctostaphylos pungens*	Chico, Cal.	11/53	l, tw	−	...	.....	++	0	++	+
3558	*Arctostaphylos pungens*	Chico, Cal.	11/53	fr	−	...	.....	+	0	0	0
3559	*Arctostaphylos pungens*	Chico, Cal.	11/53	wd	−	...	.....	0	0	+	0
3708	*Desmothamnus lucidus*	Wayne Co., Ga.	3/54	l, tw	−	...	.....	+	0	+++	+
3732	*Leucothoe axillaris*	Southport, Brunswick Co., N. C.	4/54	l, tw	−	...	.....	++	0	+++	+
3710	*Lyonia fruticosa*	Wayne Co., Ga.	3/54	l, tw	−	...	.....	++	0	+++	+
3592	*Lyonia lucida*	Southport, Brunswick Co., N. C.	2/54	l, s	−	...	.....	++	0	+	++
3631	*Permettya poeppigii*	La Paz, Bolivia	3/54	l	−	...	.....	++	0	+	+
3798	*Polycodium floridanum*	McIntosh Co., Ga.	4/54	l	−	...	.....	+	0	++	++
3799	*Vaccinium arboreum*	Long Co., Ga.	4/54	l	−	...	.....	0	0	+	++
				EUPHORBIACEAE							
3849	*Acatypha sp.*	Cava de la Lechuza, Tingo Maria, Peru	4/54	l, tw	−	...	.....	0	0	+++	++
3950	*Acatypha sp.*	Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	+++	+++
3057	*Alchornia cordifolia*	C. G. F.	9/52	l	−	...	.....	+	0	+	++
3784	*Croton punctatus*	McIntosh Co., Ga.	4/54	l, s	−	...	.....	+	0	0	+
3851	*Euphorbia sp.*	Tingo Maria, Peru	4/54	l, tw	−	...	.....	0	0	0	++
3619	*Hura crepitans*	Cochabamba, Bolivia	2/54	b	−	...	.....	0	0	0	0
3949	*Jatropha sp.*	Tingo Maria, Peru	4/54	l, s	−	...	.....	+	0	0	++
3002	*Sapium sebiferum*	C. G. F.	8/52	l	−	...	.....	0	0	0	0
				FAGACEAE							
3797	*Quercus cinerea*	Long Co., Ga.	4/54	l, s	−	...	.....	0	0	+++	++
3915	*Quercus laevis*	Southport, Brunswick Co., N. C.	4/54	l	−	...	.....	+	0	+++	+++
3930	*Quercus nigra*	Oglethorpe Co., Ga.	4/54	l, s	−	...	.....	++	0	+++	++

Accession No.	Collection: Species	Collection: Species	Collection: Date Collected	Collection: Plant Part	Hemolysis Test	Sapogenins: Total, M. F. B., %	Sapogenins: Identified, % of Total	Flavonoids	Alkaloids	Tannins	Sterols
				GERANIACEAE							
3643	*Erodium cicutarium*	Clarke Co., Ga.	3/54	l	−	...	.....	0	0	0	++
3689	*Erodium cicutarium*	Raleigh, N. C.	3/54	w	−	...	.....	+	0	0	++
3819-O	*Erodium sp.*	Greece (P. Manos)	3/54	l	−	...	.....	0	0	0	+
3686	*Geranium carolinianum*	Raleigh, N. C.	3/54	l	−	...	.....	+	0	0	+++
3770	*Geranium carolinianum*	Bullock Co., Ga.	4/54	l, s	−	...	.....	0	0	+++	+++
				GNETACEAE							
3546	*Ephedra trifurca*	H. M. Woodman, Truth or Consequence, N. Mex.	10/53	wd, l, s	+	0	.....	0	0	0	0
				GRAMINEAE							
3842	*Anthoxanthum odoratum*	Raleigh, N. C.	4/54	w	−	...	.....	0	0	0	++
3936	*Bromus catharticus*	Clarke Co., Ga.	4/54	l, s	−	...	.....	0	0	0	+++
3655	*Neyraudia madagascariensis*	C. G. F.	3/54	l, s	−	...	.....	+	0	0	++
3787	*Stipa avenacea*	McIntosh Co., Ga.	4/54	l, s	−	...	.....	0	0	0	++
3653	*Themeda gigantea var. vulpina*	C. G. F.	3/54	l, s	−	...	.....	+	0	0	++
3656	*Tripsacum laxum*	C. G. F.	3/54	l, s	−	...	.....	+	0	0	++
3651	*Zoysia japonica var. pungens*	C. G. F.	3/54	l	−	...	.....	+	0	0	++
				GUTTIFERAE							
3598	*Garcinia dulcis (cult.)*	Atkins Gard., Soledad, Cuba	2/54	l	−	...	.....	++	0	0	++
3894	*Vismia sp.*	Tingo Maria, Peru	4/54	l, tw, b	−	...	.....	+	0	+++	+
				HAMAMELIDACEAE							
3796	*Liquidambar styraciflua*	McIntosh Co., Ga.	4/54	l, s	−	...	.....	+	0	++	++
				HIPPOCASTANACEAE							
3777	*Aesculus pavia*	Greene Co., Ga.	4/54	l, s, r	−	...	.....	++	0	+++	++
3734	*Aesculus pavia*	Raleigh, N. C.	4/54	l, tw	+	0	.....	+	0	0	+++
3679	*Aesculus sylvaticus*	Raleigh, N. C.	3/54	l, tw	−	...	.....	0	0	+++	+
				JUGLANDACEAE							
3012	*Pterocarya stenoptera*	C. G. F.	8/52	l	−	...	.....	++	0	0	0
				JULIANACEAE							
3144	*Juliania adstringens*	Camaron, Oax., Mex.	9/52	b	+	0	.....	+	0	0	0
3218	*Juliania glauca*	N. W. of Apatzingan, Michoa., Mex.	10/52	b	+	0	.....	+	0	++	0
				LABIATAE							
3642	*Lamium amplexicaule*	Clarke Co., Ga.	3/54	l	−	...	.....	+	0	0	+
3687	*Lamium amplexicaule*	Raleigh, N. C.	3/54	w	−	...	.....	0	0	0	++
3645	*Lamium purpureum*	Clarke Co., Ga.	3/54	l	−	...	.....	+	0	0	++
3779	*Salvia lyrata*	McIntosh Co., Ga.	4/54	l, s	−	...	.....	0	0	0	++
3947	*Scutellaria speciosa*	W. of Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	+
				LAURACEAE							
3789	*Cinnamomum camphora*	McIntosh Co., Ga.	4/54	l	−	...	.....	0	0	0	+++
3615	*Persea borbonia*	Southport, Brunswick Co., N. C.	2/54	l	−	...	.....	+	0	0	+

Accession No.	Species	Collection Source	Date Collected	Plant Part	Hemolysis Test	Sapogenins Total, M. F. B., %	Sapogenins Identified, % of Total	Flavonoids	Alkaloids	Tannins	Sterols
				LAURACEAE							
3706	*Persea pubescens*	Wayne Co., Ga.	3/54	l, s	−	...	.....	+	0	0	++
				LEGUMINOSAE							
3032	*Acacia caesia*	C. G. F.	9/52	l, fr	−	...	.....	0	0	0	++
3029	*Acacia moniliformis*	C. G. F.	9/52	l	−	...	.....	+	0	0	+++
3013	*Albizzia brownii*	C. G. F.	8/52	l	−	...	.....	+	0	0	0
3602	*Albizzia polyphylla (cult.)*	Atkins Gard., Soledad, Cuba	2/54	l, s, fr	−	...	.....	++	++	0	++
3909	*Amorpa fruticosa*	Southport, Brunswick Co., N. C.	4/54	l, s, tw	−	...	.....	0	0	0	+++
3720	*Baptisia lanceolata*	Wayne Co., Ga.	3/54		−	...	.....	+	+	0	+
3719	*Baptisia perfoliata*	Toombs Co., Ga.	3/54	l	−	...	.....	+	+	0	+
3006	*Caesalpinia vesicaria*	C. G. F.	8/52	l	−	...	.....	+	0	0	0
3009	*Cassia baccilarias*	C. G. F.	8/52	l	−	...	.....	0	0	0	0
3898	*Crotalaria sp.*	Exp. Sta., Tingo Maria, Peru	4/54	l, s	+	0	.....	++	0	0	+
3688	*Cytisus scoparius*	Raleigh, N. C.	3/54	tw	−	...	.....	+	+++	0	++
3886	*Desmodium intortum*	Mt. Divisoria, Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	++
3028	*Erythrina senegalensis*	C. G. F.	9/52	l	−	...	.....	0	0	0	0
3862	*Indigofera sp.*	Rd. to Puerto Nuevo, Tingo Maria, Peru	4/54	l, tw	+	0	.....	0	0	0	+
3867	*Inga sp.*	Tingo Maria, Peru	4/54	l, tw, b	−	...	.....	0	0	++	++
3010	*Lonchocarpus latifolius*	C. G. F.	8/52	l	−	...	.....	0	0	0	0
3718	*Lupinus diffusus*	Tattnall Co., Ga.	3/54	l	−	...	.....	0	+++	0	+++
3716	*Lupinus villosus*	Wayne Co., Ga.	3/54	l	−	...	.....	+	+++	0	+++
3254	*Lysiloma candida*	Mulj, B. C. Mex.	12/52	b	+	0	.....	+	0	+++	+
3215	*Lysiloma divaricata*	San Bernardo, Son., Mex.	10/52	b	+	0	.....	+	0	+++	++
3216	*Lysiloma watsonii*	San Bernardo, Son., Mex.	10/52	b	+	0	.....	+	0	0	0
3464	*Lysiloma watsonii*	San Bernardo, Son., Mex.	4/53	b	−	...	.....	0	0	+++	0
3081	*Phaseolus coccineus*	S. of Miahautlan, Oax., Mex.	9/52	r	+	0.3 mi	.....	0	0	0	0
3109	*Phaseolus vulgaris*	G. D. M.	9/52	l, s	−	...	.....	0	0	0	+
3055	*Piptadenia rigida*	C. G. F.	9/52	l	−	...	.....	+	0	++	++
3843	*Pisum sativum var. arvense*	Raleigh, N. C.	4/54	l, tw	−	...	.....	+	0	0	++
3217	*Pithecellobium mexicanum*	San Bernardo, Son., Mex.	10/52	s, r	+	0	.....	0	0	0	0
3924	*Robinia pseudo-acacia*	Clarke Co., Ga.	4/54	l	−	...	.....	0	0	0	++
3920	*Trifolium arvense*	Southport, Brunswick Co., N. C.	4/54	w	−	...	.....	0	0	0	+
3776	*Vicia angustifolia*	Clarke Co., Ga.	4/54	l, s	−	...	.....	0	0	0	+++
3839	*Vicia grandiflora*	Raleigh, N. C.	4/54	w	−	...	.....	+	0	0	++
3911	*Wisteria frutescens*	Southport, Brunswick Co., N. C.	4/54	l, tw	−	...	.....	+	0	0	+++
3919	*Wisteria sinensis*	Southport, Brunswick Co., N. C.	4/54	l	−	...	.....	+	0	0	+++
				LILIACEAE							
3120-O	*Aloe vera*	Aruba (C. H. G. Eman)	10/52	l	−	...	.....	0	0	0	0

Accession No.	Species	Collection: Species	Date Collected	Plant Part	Heinol-ysis Test	Sapogenins: Total, M. F. B., %	Sapogenins: Identified, % of Total	Flavonoids	Alkaloids	Tannins	Sterols
				LILIACEAE							
3121-O	*Aloe vera*	Aruba (C. H. G. Eman)	10/52	Dried juice	+	0.8 mi	.....	0	0	0	0
3017	*Anthericum sp.*	W. of Igula, Guer., Mex.	8/52	w	+	0	.....	0	0	0	0
3213	*Asphodeline sp.*	Bovnova, Turkey	10/52	t	+	0	.....	0	0	0	+
3197	*Beaucarnea inermis*	Manuel, Tam., Mex.	10/52	s	−	...	.....	0	0	0	+
3337	*Eucomis undulata*	G. D. M.	1/53	r, bu, l	+	0	.....	0	0	0	0
3712	*Liriope spicata*	Wayne Co., Ga.	3/54	l	−	...	.....	0	0	0	++
3335	*Ornithogalum sp.*	G. D. M.	12/52	r, bu, l	+	0	.....	0	0	0	+
3336	*Ornithogalum sp.*	G. D. M.	12/52	r, bu, l	+	0	.....	0	0	0	+
3453	*Smilax sp.*	Cuyotenango, Dept. Such., Guat.	3/53	r	−	...	.....	0	0	+++	0
3416	*Smilax sp.*	N. W. of Cofradia, Hond.	3/53	r	−	...	.....	0	0	++	0
3401	*Urginia burkei*	Transvaal	2/53	bu	−	...	.....	0	0	++	+
3586A	*Yucca filmentosa*	Southport, Brunswick Co., N. C.	2/54	l	+	0.3	k100	..	..	..	..
3586B	*Yucca filamentosa*	Southport, Brunswick Co., N. C.	2/54	r	+	0	.....	..	..	..	..
3527	*Yucca gloriosa*	Southport, Brunswick Co., N. C.	7/53	l	+	0	.....	..	..	..	..
3528	*Yucca gloriosa*	Southport, Brunswick Co., N. C.	7/53	l	+	0	.....	..	..	..	..
3265	*Yucca peninsularis*	Sierra San Miguel, B. C., Mex.	12/52	l, dead	+	2.5 ir	t; h	..	..	..	..
3274	*Yucca peninsularis*	Laguna Jaraguay, B. C., Mex.	12/52	l	+	1.0 ir	t	..	..	..	..
3280	*Yucca peninsularis*	Sierra San Miguel, B. C., Mex.	12/52	l, dead	+	1.1 ir	t; g, h	..	..	..	..
3332	*Yucca peninsularis*	San Miguel, B. C., Mex.	12/52	l	+	0.3 ir	t; h, g	..	..	..	..
3515	*Yucca peninsularis*	N. of San Fernando, B. C., Mex.	5/53	l	+	1.3	t80; g20	..	..	..	..
3516	*Yucca peninsularis*	N. of San Fernando, B. C., Mex.	5/53	l	+	1.4 ir	t; g	..	..	..	..
3305	*Yucca schidigera*	N. of San Fernando, B. C., Mex.	12/52	l	+	0.9 ir	ss; mk	..	..	..	..
3244	*Yucca schidigera*	Aguanga, Cal.	12/52	l	+	1.3	ss100	..	..	..	..
3223-O	*Yucca schidigera*	Kingman, Ariz.	11/52	Juice	+	83 mg/gal.	ss100	..	..	..	..
3480	*Yucca schidigera*	Kingman, Ariz.	4/53	l	+	3.6 ir	ss	..	..	..	..
3224	*Yucca whipplei*	Murrieta, Cal.	11/52	l, dead	+	1.1	t80; g20	..	..	..	..
3225	*Yucca whipplei*	Murrieta, Cal.	11/52	l, green	+	0.9	t100	..	..	..	..
3399	*Yucca whipplei*	Calif.	2/53	l	+	1.0 ir	t	..	..	..	..
3253	*Yucca sp.*	B. C., Mex.	12/52	l	+	0.9	t100	..	..	..	..
3395	*Yucca sp.*	Kingman, Ariz.	2/53	l	−	...	.....	..	..	..	..
3497	*Yucca sp.*	Djakarta, Indonesia	5/53	l, juice	+	0	.....	..	..	..	..
3498	*Yucca sp.*	Djakarta, Indonesia	5/53	l, juice	+	0.2 mi	.....	..	..	..	..
				LINACEAE							
3582	*Linum usitatissimum*	Corvallis, Ore.	2/54	Straw	+	0	.....	++	0	0	+
3583	*Linum usitatissimum*	Corvallis, Ore.	2/54	sd	+	0	.....	0	0	0	0

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				LOASACEAE							
3900	*Loasa sp.*	Quilca, Lima, Peru	4/54	l, s	−	...	.....	0	0	0	+
				LOGANIACEAE							
3587	*Gelsemium sempervirens*	Southport, Brunswick Co., N. C.	2/54	l, s	−	...	.....	++	0	0	++
				LYTHRACEAE							
3999	*Heimia sp.*	Rioo Chinchao, Tingo Maria, Peru	4/54	l, s, b	−	...	.....	0	0	+++	+
				MAGNOLIACEAE							
3593	*Magnolia virginiana*	Southport, Brunswick Co., N. C.	2/54	l, s	−	...	.....	++	0	0	++
				MALPIGHIACEAE							
3214	*Mascagnia macroptera*	San Bernando, Son.. Mex.	10/52	b	+	0	....	+	0	+++	++
3444	*Stigmaphyllon humboldtianum*	G. D. M.	3/53	r, t	−	...	.....	0	0	0	0
3445	*Stigmaphyllon humboldtianum*		3/53	r, t	−	...	.....	0	0	0	0
3203	*Stigmaphyllon selerianum*	Balsa Larga, Ver., Mex.	10/52	r	+	0	.....	+	0	0	0
3502	*Stigmaphyllon sp.*	Martinez de la Torre, Ver., Mex.	5/53	r	+	0	.....	0	0	0	0
				MALVACEAE							
3780	*Modiola caroliniana*	McIntosh Co., Ga.	4/54	l, s	+	0	.....	0	0	0	+++
3845	*Sida sp.*	Tingo Maria, Peru	4/54	l, tw	−	...	.....	+	0	0	++
3885	*Sida sp.*	Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	++
				MELASTOMATACEAE							
3850	*Blakea mexiae*	Puento Prado, Tingo Maria, Peru	4/54	l, tw, b	−	...	.....	0	0	+++	++
3977	*Clidemia dentata*	E. of Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	+++	++
3970	*Leandra dichotoma*	Monzon Rd., Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	+++
3882	*Loreya mespiloides*	E. of Tingo Maria, Peru	4/54	l, tw, b	−	...	.....	0	0	+	+++
3855	*Miconia sp.*	E. of Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	++
3864	*Miconia sp.*	W. of Tingo Maria, Peru	4/54	l, s, b	−	...	.....	0	0	+++	++
3892	*Miconia sp.*	Bet. Divisoria and Carmen Alto, Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	++	++
3893	*Miconia sp.*	Bet. Divisoria and Carmen Alto, Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	++
				MENISPERMACEAE							
3106	*Stephania cephalantha*	G. D. M.	9/52	l, s, t	−	...	.....	0	++	0	+
				MORACEAE							
3968A	*Brosimum sp.*	Monzon Rd., Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	+++

MORACEAE

3968B	*Brosimum sp.*	Monzon Rd., Tingo Maria, Peru	4/54	b	–	...	.....	0	0	0	0
3036	*Broussonetia papyrifera*	C. G. F.	9/52	l	–	...	.....	0	0	0	0
3670A	*Ficus altissima*	C. G. F.	3/54	l, s, r	–	...	.....	0	0	0	++
3670B	*Ficus altissima*	C. G. F.	3/54	fr	–	...	.....	0	0	0	+
3605	*Ficus archeri*	C. G. F.	2/54	l, s	–	...	.....	0	0	0	++
3613A	*Ficus asperrima*	C. G. F.	2/54	l, s	–	...	.....	0	0	0	+
3613B	*Ficus asperrima*	C. G. F.	2/54	fr	+	0	.....	0	0	0	+
3606	*Ficus benghalensis*	C. G. F.	2/54	l, s, r	–	...	.....	0	0	0	++
3660	*Ficus brevifolia*	C. G. F.	3/54	l, s, fr	–	...	.....	+	0	0	+
3661	*Ficus bussei*	C. G. F.	3/54	l, s	–	...	.....	+	0	0	++
3607	*Ficus calophylloides*	C. G. F.	2/54	l, s, r	–	...	.....	0	0	0	++
3696	*Ficus capensis*	C. G. F.	3/54	l, s, fr	–	...	.....	0	0	0	+
3626	*Ficus caudatifolia*	C. G. F.	3/54	l, s	–	...	.....	+	0	0	++
3695	*Ficus columnaris*	C. G. F.	3/54	l, s	–	...	.....	0	0	0	++
3674A	*Ficus dumosa*	C. G. F.	3/54	l, s, r	–	...	.....	0	0	0	++
3674B	*Ficus dumosa*	C. G. F.	3/54	fr	–	...	.....	0	0	0	+
3819	*Ficus dusenii*	C. G. F.	4/54	l, tw	–	...	.....	0	0	0	0
3700	*Ficus elastica*	C. G. F.	3/54	l, s, fr, r	–	...	.....	0	0	0	+
3702	*Ficus eriobotryoides*	C. G. F.	3/54	l, s, fr, r	–	...	.....	0	0	0	0
3621A	*Ficus eugenioides*	C. G. F.	3/54	l, s, r	–	...	.....	0	0	0	+
3621B	*Ficus eugenioides*	C. G. F.	3/54	fr	+	0	.....	0	0	0	+
3608	*Ficus gibbosa*	C. G. F.	2/54	l, s	–	...	.....	0	0	0	++
3697	*Ficus glomerata*	C. G. F.	3/54	l, s	–	...	.....	0	0	0	+
3672	*Ficus gnaphalocarpa*	C. G. F.	3/54	l, s, r	–	...	.....	++	0	0	++
3623	*Ficus henneana*	C. G. F.	3/54	l, s	–	...	.....	0	0	0	++
3814	*Ficus hookeri*	C. G. F.	4/54	l, tw, r	–	...	.....	0	0	0	0
3614	*Ficus jacquinifolia*	C. G. F.	2/54	l, s, r	–	...	.....	0	0	0	0
3610	*Ficus krishnae*	C. G. F.	2/54	l, s	–	...	.....	0	0	0	++
3662	*Ficus lacor*	C. G. F.	3/54	l, s, r	–	...	.....	+	0	0	++
3609	*Ficus laevigata*	C. G. F.	2/54	l, s	–	...	.....	0	0	0	++
3817	*Ficus macrophylla*	C. G. F.	4/54	l, tw	–	...	.....	0	0	0	+
3816	*Ficus mariannensis*	C. G. F.	4/54	l, tw, f, r	–	...	.....	0	0	+	+
3699	*Ficus minakassae*	C. G. F.	3/54	l, s	–	...	.....	0	0	0	0
3693	*Ficus mitrophora*	C. G. F.	3/54	l, s, r	–	...	.....	0	0	0	+
3625	*Ficus mysorensis*	C. G. F.	3/54	l s, r	–	...	.....	0	0	0	+
3701	*Ficus nitida*	C. G. F.	3/54	l, s, fr, r	–	...	.....	0	0	0	+
3663	*Ficus nota*	C. G. F.	3/54	l, s	–	...	.....	0	0	0	++
3627	*Ficus odorata*	C. G. F.	3/54	s	–	...	.....	0	0	0	0
3624	*Ficus padifolia*	C. G. F.	4/54	l, s	–	...	.....	0	0	0	+
3815	*Ficus padifolia*	C. G. F.	4/54	l, tw, r	–	...	.....	0	0	0	0
3667	*Ficus palmata*	C. G. F.	3/54	l, s	–	...	.....	0	0	0	++
3664	*Ficus pandurata*	C. G. F.	3/54	l, s	–	...	.....	0	0	++	++
3612	*Ficus platyphylla*	C. G. F.	2/54	l, s, r	–	...	.....	0	0	0	+
3668A	*Ficus platypoda var. petiolaris*	C. G. F.	3/54	l, s, r	–	...	.....	0	0	0	++
3668B	*Ficus platypoda var. petiolaris*	C. G. F.	3/54	fr	–	...	.....	0	0	0	+
3671A	*Ficus pretoriae*	C. G. F.	3/54	l, s	–	...	.....	++	0	0	++
3671B	*Ficus pretoriae*	C. G. F.	3/54	fr	–	...	.....	0	0	0	+

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				MORACEAE							
3698	*Ficus pumila*	C. G. F.	3/54	l, s	−	...	.....	0	0	0	+
3665	*Ficus religiosa*	C. G. F.	3/54	l, s	−	...	.....	0	0	0	+
3703	*Ficus rigo*	C. G. F.	3/54	l, s, fr	−	...	.....	0	0	0	0
3622	*Ficus roxburghii*	C. G. F.	3/54	l, s, r	−	...	.....	++	0	0	+
3611A	*Ficus rubiginosa*	C. G. F.	2/54	l, s	−	...	.....	0	0	0	++
3611B	*Ficus rubiginosa*	C. G. F.	2/54	fr	+	0	.....	0	0	0	+
3620	*Ficus salicifolia*	C. G. F.	3/54	l, s, r	−	...	.....	0	0	0	++
3669	*Ficus sycomorus*	C. G. F.	3/54	l, s, fr, r	−	...	.....	+	0	0	++
3704	*Ficus tinctoria*	C. G. F.	3/54	l, s, fr	−	...	.....	0	0	0	++
3694	*Ficus ulmifolia*	C. G. F.	3/54	l, s	−	...	.....	+	0	0	+
3666	*Ficus vogelii*	C. G. F.	3/54	l, s, fr	−	...	.....	0	0	0	++
3818	*Ficus volkensii*	C. G. F.	4/54	l, tw	−	...	.....	0	0	+	+
3628A	*Ficus watkinsiana*	C. G. F.	3/54	l, s	−	...	.....	+	0	0	+
3628B	*Ficus watkinsiana*	C. G. F.	3/54	fr	+	0	.....	0	0	0	0
3628C	*Ficus watkinsiana*	C. G. F.	3/54	r	−	...	.....	+	0	0	+
3982	*Ficus sp.*	Tingo Maria, Peru	4/54	l, tw, b	−	...	.....	+	0	0	++
3320	*Ficus sp.*	Mexico City, Mex.	12/52	latex	−	...	.....	..	..	..	..
3321	*Ficus sp.*	Mexico City, Mex.	12/52	latex	−	...	.....	..	..	..	..
				MUSACEAE							
3992	*Heliconia tenebrosa*	Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	0
				MYRICACEAE							
3588	*Myrica cerifera*	Southport, Brunswick Co., N. C.	2/54	l, s	−	...	.....	++	0	+++	++
3707	*Myrica cerifera*	Wayne Co., Ga.	3/54	l	−	...	.....	++	0	++	++
3638	*Myrica rubra (cult.)*	Atkins Gard., Soledad, Cuba	3/54	l, s	−	...	.....	++	0	++	+
				MYRTACEAE							
3058	*Eucalyptus alba*	C. G. F.	9/52	l, fr	−	...	.....	+	0	0	++
3063	*Eucalyptus camaldulensis*	C. G. F.	9/52	l, fr	−	...	.....	+	0	0	+
3088	*Eucalyptus citriodora*	C. G. F.	9/52	l, fr	−	...	.....	+	0	0	++
3091	*Eucalyptus crebra*	C. G. F.	9/52	l, fr	−	...	.....	+	0	0	0
3094	*Eucalyptus crebra*	C. G. F.	9/52	l, fr	−	...	.....	+	0	+	0
3093	*Eucalyptus exserta*	C. G. F.	9/52	l, fr	−	...	.....	+	0	+	+
3574	*Eucalyptus globulus*	Arboretum, Arcadia, Cal.	12/53	sd	−	...	.....	0	0	+++	+
3085	*Eucalyptus haemastoma*	C. G. F.	9/52	l, fr	−	...	.....	+	0	+++	++
3065	*Eucalyptus kirtoniana*	C. G. F.	9/52	l, fr	−	...	.....	+	0	++	+
3060	*Eucalyptus longifolia*	C. G. F.	9/52	l, fr	−	...	.....	+	0	++	++
3086	*Eucalyptus obliqua*	C. G. F.	9/52	l, fr	−	...	.....	+	0	+++	0
3084	*Eucalyptus oranensis*	C. G. F.	9/52	l, fl, fr	−	...	.....	+	0	+	++
3062	*Eucalyptus paniculata*	C. G. F.	9/52	l, fr	−	...	.....	0	0	0	0
3087	*Eucalyptus pauciflora*	C. G. F.	9/52	l, fr	−	...	.....	+	0	+	+
3089	*Eucalyptus paulistana*	C. G. F.	9/52	l, fr	−	...	.....	+	0	+	0
3092	*Eucalyptus polyanthemos*	C. G. F.	9/52	l, fr	−	...	.....	+	0	0	+

			MYRTACEAE								
3090	*Eucalyptus punctata*	C. G. F.	9/52 l, fr	–	...	.....	+	0	+	++	
3096	*Eucalyptus regnans*	C. G. F.	9/52 l, fr	–	...	.....	+	0	+	0	
3097	*Eucalyptus regnans*	C. G. F.	9/52 l, fr	–	...	.....	+	0	+	++	
3061	*Eucalyptus resinifera*	C. G. F.	9/52 l, fr	–	...	.....	+	0	+	0	
3064	*Eucalyptus robusta*	C. G. F.	9/52 l, fr	–	...	.....	+	0	+	+	
3059	*Eucalyptus rudis*	C. G. F.	9/52 l, fr	–	...	.....	+	0	0	++	
3095	*Eucalyptus triantha*	C. G. F.	9/52 l, fr	–	...	.....	+	0	++	0	
3026	*Eugenia atropunctata*	C. G. F.	9/52 l, fr	–	...	.....	++	0	0	++	
3603	*Syzygium lineatum (cult.)*	Atkins Gard., Soledad, Cuba	2/54 l, s	–	...	.....	0	0	+	++	
			OLACACEAE								
3119	*Ptychopetalum olacoides*	Belem, Brazil	9/52 r	+	0	.....	0	0	0	0	
			OLEACEAE								
3912	*Chionanthus virginica*	Southport, Brunswick Co., N. C.	4/54 l, fl	–	...	.....	0	0	0	+++	
3832	*Ligustrum lucidum*	Raleigh, N. C.	4/54 l, tw	–	...	.....	+	0	0	+++	
3844	*Ligustrum vulgare*	Raleigh, N. C.	4/54 l, tw	–	...	.....	+	0	0	++	
3813	*Ligustrum sinense*	Athens, Ga.	4/54 l, s	–	...	.....	0	0	0	++	
3594	*Osmanthus americanus*	Southport, Brunswick Co., N. C.	2/54 l, s	–	...	.....	++	0	0	+	
3713	*Osmanthus americanus*	Wayne Co., Ga.	3/54 l	–	...	.....	+	0	0	++	
			ONAGRACEAE								
3887	*Fuchsia abrupta*	E. of Divisoria, Tingo Maria, Peru	4/54 l, s	–	...	.....	0	0	+++	++	
3895	*Jussiaea sp.*	Tingo Maria, Peru	4/54 l, s	–	...	.....	+	0	+++	+	
3729	*Oenothera humifusa*	Raleigh, N. C.	3/54 w	–	...	.....	++	0	0	++	
3685	*Oenothera laciniata*	Raleigh, N. C.	3/54 l	–	...	.....	0	0	0	+++	
3935	*Oenothera speciosa*	Athens, Ga.	4/54 l, s	–	...	.....	+++	0	0	++	
			OSMUNDACEAE								
3917	*Osmunda cinnamomea*	Southport, Brunswick Co., N. C.	4/54 l	–	...	.....	0	0	0	++	
3916	*Osmunda regalis var. spectabilis*	Southport, Brunswick Co., N. C.	4/54 l	–	...	.....	0	0	0	+++	
			OXALIDACEAE								
3053	*Oxalis sp.*	Tepoztlan, Morel, Mex.	9/52 w	+	0	.....	0	0	0	0	
			PALMAE								
3652	*Acanthorhiza aculeata*	C. G. F.	3/54 l, s	+	0	.....	+	0	0	++	
3654	*Acrocomia sclerocarpa*	C. G. F.	3/54 l, s. fr	–	...	.....	++	0	0	++	
3563	*Cocos australis*	Chico, Cal.	11/53 l	–	...	.....	0	0	0	++	
3560	*Phoenix canariensis*	Chico, Cal.	11/53 l	–	...	.....	+	0	0	++	
3561	*Phoenix canariensis*	Chico, Cal.	11/53 Spadix	–	...	.....	0	0	0	+	
3562	*Phoenix canariensis*	Chico, Cal.	11/53 fr	–	...	.....	0	0	0	+	
3659	*Pseudophoenix vinifera*	C. G. F.	3/54 Petiole	–	...	.....	++	0	0	+	
			PAPAVERACEAE								
3786	*Argemone alba*	McIntosh Co., Ga.	4/54 l, s	–	...	.....	0	0	0	++	

Accession No.	Species	Collection: Source	Date Collected	Plant Part	Hemolysis Test	Sapogenins: Total M. F. B. %	Sapogenins: Identified, % of Total	Flavonoids	Alkaloids	Tannins	Sterols
3733	*Corydalis micrantha*	Raleigh, N. C.	4/54	w	−	...	.....	0	+	0	+++
				PHYTOLACCACEAE							
3517	*Limeum aethiopicum*	G. D. M.	6/53	t	+	0	.....	0	0	0	0
3969	*Phytolacca sp.*	Monzon Rd., Tingo Maria, Peru	4/54	l, s, sd	+	0	.....	0	0	0	+
				PINACEAE							
3276	*Stegnosperma halimifolium*	Santa Rosalia, B. C., Mex.	12/52	l	+	0	.....	0	0	0	+
3640A	*Cupressus funebris (cult.)*	Atkins Gard., Soledad, Cuba	3/54	l, s	−	...	.....	++	0	0	+
3640B	*Cupressus funebris (cult.)*	Atkins Gard., Soledad, Cuba	3/54	b	−	...	.....	0	0	0	+
3677	*Juniperus virginiana*	Raleigh, N. C.	3/54	l, tw	−	...	.....	+	0	++	++
3591	*Pinus palustris*	Southport, Brunswick Co., N. C.	2/54	l	−	...	.....	+	0	0	++
3678	*Pinus taeda*	Raleigh, N. C.	3/54	l, tw	−	...	.....	+	0	+	++
				PIPERACEAE							
3988	*Piper sp.*	E. of Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	+++
3963	*Piper sp.*	Rio Monzon, Tingo Maria, Peru	4/54	l, s, b	−	...	.....	0	0	0	+++
				PITTOSPORACEAE							
3723	*Pittosporum tobira*	Raleigh, N. C.	4/54	l, tw	−	...	.....	+	0	0	++
				PLANTAGINACEAE							
3684	*Plantago lanceolata*	Raleigh, N. C.	3/54	w	−	...	.....	0	0	0	+++
3764	*Plantago lanceolata*	Oglethorpe Co., Ga.	4/54	l	−	...	.....	0	0	0	++
				POLYGONACEAE							
3925	*Polygonum cuspidatum*	Athens, Ga.	4/54	l, s, r	−	...	.....	0	0	0	++
3641	*Rumex crispus*	Athens, Ga.	3/54	l, s	−	...	.....	0	0	0	+
3767	*Rumex hastatulus*	Oglethorpe Co., Ga.	4/54	l, s	−	...	.....	0	0	0	+
3682	*Rumex obtusifolius*	Raleigh, N. C.	3/54	l, r	−	...	.....	0	0	0	+++
				POLYPODIACEAE							
3794	*Asplenium platyneuron*	McIntosh Co., Ga.	4/54	l	−	...	.....	0	0	0	++
3793	*Dryopteris normalis*	McIntosh Co., Ga.	4/54	l	−	...	.....	0	0	0	++
3681	*Polystichum arostichoides*	Raleigh, N. C.	3/54	w	+	0	.....	++	0	0	+++
3803	*Pteridium aquilinum* var. *pseudocaudatum*	Toombs Co., Ga.	4/54	l	−	...	.....	0	0	0	+++
3714	*Woodwardia areolata*	Wayne Co., Ga.	3/54	l	−	...	.....	0	0	0	++
3802	*Woodward virginica*	Toombs Co., Ga.	4/54	l, s	−	...	.....	0	0	+++	+++
				RANUNCULACEAE							
3830	*Ranunculus abortivus*	Raleigh, N. C.	4/54	w	−	...	.....	+	0	0	+++
3809	*Ranunculus abortivus*	Athens, Ga.	4/54	l	−	...	.....	0	0	0	+
3932	*Ranunculus parviflorus*	Athens, Ga.	4/54	l, s	−	...	.....	0	0	0	++
3928	*Ranunculus recurvatus*	Oglethorpe Co., Ga.	4/54	l, s	−	...	.....	0	0	0	++
				RHAMNACEAE							
3465	*Karwinskya humboldtiana*	San Bernardo, Son., Mex.	4/53	l	−	...	.....	+++	0	0	+

			RHIZOPHORACEAE								
3066	*Rhizophora mangle*	Mex. (J. W. Ayers)	9/52	b	+	0	.....	+	0	+	0
			ROSACEAE								
3836	*Amelanchier arborea*	Raleigh, N. C.	4/54	l, tw	−	...	.....	++	0	0	+++
3459	*Crataegus monogyna*	Afyon, Turkey	10/52	sd	−	...	.....	0	0	0	+
3460	*Crataegus sp.*	Eskisehir, Turkey	10/52	sd	−	...	.....	0	0	0	+
3117	*Crataegus sp.*	Taysi Yozgat, Turkey	8/52	sd	−	...	.....	0	0	0	+
3458	*Crataegus sp.*	Malatya, Turkey	9/52	sd	−	...	.....	0	0	0	0
3807	*Duchesnea indica*	Clarke Co., Ga.	4/54	l, s, fr	−	...	.....	0	0	0	++
3812	*Potentilla canadensis*	Oglethorpe Co. Ga.	4/54	l	−	...	.....	0	0	0	+
3118	*Poterium sanquisorba*	Endicott, N. Y. (R. L. Eisworth)	9/52	w	−	...	.....	0	0	0	0
3931	*Prunus angustifolia*	Athens, Ga.	4/54	l, s	−	...	.....	+	0	++	+++
3838	*Prunus serotina*	Raleigh, N. C.	4/54	l, t, w, fl	−	...	.....	+	0	0	+++
3927	*Prunus serotina*	Winterville, Oglethorpe Co., Ga.	4/54	l, s	−	...	.....	++	0	0	++
			RUBIACEAE								
3996	*Calycophyllum sp.*	Rio Chincha, Tingo Maria, Peru	4/54	l, s, b	−	...	.....	0	0	0	+
3673A	*Coffea arabica*	C. G. F.	3/54	l, s, r	−	...	.....	0	0	0	++
3673B	*Coffea arabica*	C. G. F.	3/54	fr	−	...	.....	0	0	0	0
3846	*Condaminea corymbosa*	Monzon Riv., Tingo Maria, Peru	4/54	l, tw, b	−	...	.....	0	0	0	++
3774	*Galium aparine*	Clarke Co., Ga.	4/54	l, s	−	...	.....	0	0	0	++
3858A	*Gonzalagunia cornifolia*	Huallaga Riv., Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	+++	++
3858B	*Gonzalagunia cornifolia*	Huallaga Riv., Tingo Maria, Peru	4/54	b	−	...	.....	0	0	0	+
3944	*Hamelia patens*	W. of Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	+++
3881	*Isertia sp.*	E. of Tingo Maria, Peru	4/54	l, tw, b	−	...	.....	0	0	+++	+
3595	*Ixora acuminata* (*cult.*)	Atkins Gard., Soledad, Cuba	2/54	l	−	...	.....	0	0	0	++
3596	*Ixora macrothyrsa* (*cult.*)	Atkins Gard., Soledad, Cuba	2/54	l, s, fl	−	...	.....	+	0	0	++
3027	*Portlandia domingensis*	C. G. F.	9/52	l	−	...	.....	0	0	0	+
3978	*Psychotria brachiata*	E. of Tingo Maria, Peru	4/54	l, s, b	+	0	.....	0	0	0	+++
3957	*Psychotria sp.*	Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	+++
			RUTACEAE								
3649-OA	*Citrus paradisi* (grapefruit)	Winter Haven, Fla.	3/54	sd	+	0	.....	0	0	0	0
3649-OB	*Citrus sinensis* (sweet orange)	Winter Haven, Fla.	3/54	sd	+	0	.....	0	0	0	0
3649-OC	*Citrus reticulata* (tangerine)	Winter Haven, Fla.	3/54	sd	+	0	.....	0	0	0	0
			SALICACEAE								
3910	*Salix caroliniana*	Southport, Brunswick Co., N. C.	4/54	l, tw, fr	−	...	.....	0	0	0	+++
			SAPINDACEAE								
3003	*Dodonaea viscosa*	C. G. F.	8/52	l	−	...	.....	0	0	0	+
3334	*Dodonaea viscosa*	G. D. M.	12/52	l	+	0	.....	+	0	0	+
3639	*Euphoria longana* (*cult.*)	Atkins Gard., Soledad, Cuba	3/54	l, s	−	...	.....	+	0	++	+

Accession No.	Species	Collection Source	Date Collected	Plant Part	Hemolysis Test	Sapogenins Total, M. F. B., %	Sapogenins Identified, % of Total	Flavonoids	Alkaloids	Tannins	Sterols
				SAPOTACEAE							
3783	*Bumelia tenax*	McIntosh Co., Ga.	4/54	l, s	+	0	.....	0	0	0	++
3650A	*Chrysophyllum olivaeformis*	Ciego de Avila, Camaguey Prov., Cuba	3/54	l, s	−	...	.....	+	0	0	++
3650B	*Chrysophyllum olivaeformis*	Ciego de Avila, Camaguey Prov., Cuba	3/54	b	−	...	.....	0	0	0	+
3604	*Madhuca indica (cult.)*	Atkins Gard., Soledad, Cuba	2/54	l, s	−	...	.....	+	0	0	++
3033	*Mimusops zeyheri*	C. G. F.	9/52	l	−	...	.....	+	0	0	+
				SCROPHULARIACEAE							
3897	*Calceolaria sp.*	Bet. Divisoria and Carmen Alto, Tingo Maria, Peru	4/54	l, s	−	...	.....	++	0	0	++
3717	*Linaria canadensis*	Wayne Co., Ga.	3/54	l	−	...	.....	0	0	0	+++
3901	*Galvesia suffruticosa*	Quilca, Lima, Peru	4/54	l, s	−	...	.....	0.	0	0	+
3692	*Verbascum thapsus*	Raleigh, N. C.	3/54	l	−	...	.....	0	0	0	++
3938	*Veronica hederaefolia*	Athens, Ga.	4/54	l, s	−	...	.....	0	0	0	+++
				SIMAROUBACEAE							
3675	*Ailanthus altissima*	Raleigh, N. C.	3/54	tw	−	...	.....	0	0	0	++
				SOLANACEAE							
3905	*Dunalia campanulata*	Quilca, Lima, Peru	4/54	l, s	+	0	.....	0	0	0	+
3896	*Nicandra physalodes*	Bet. Divisoria and Carmen Alto, Tingo Maria, Peru	4/54	l, s	+	0	.....	0	0	0	++
3548	*Solanum sp.*	Savannah, Ga.	10/53	l	−	...	.....	0	0	0	++
3618	*Solanum sp.*	Todos Santos, Cochabamba, Boliv.	2/54	r, t	−	...	.....	0	0	0	0
3939	*Solanum sp.*	Exp. Sta., Tingo Maria, Peru	4/54	l, s	−	...	.....	0	0	0	++
3943	*Solanum sp.*	Exp. Sta., Tingo Maria, Peru	4/54	l, s	−	...	.....	0	++	0	+++
				STEMONACEAE							
3493A	*Stemona sp.*	Djakarta, Indonesia	5/53	l (juice)	+	0	.....	..	..	..	..
3493B	*Stemona sp.*	Djakarta, Indonesia	5/53	w (juice)	+	0	.....	0	0	0	+
3494	*Stemona sp.*	Djakarta, Indonesia	5/53	w (juice)	+	0	.....	0	0	0	+
				SYMPLOCACEAE							
3772	*Symplocos tinctoria*	Emanual Co., Ga.	4/54	l, s	+	0	.....	+	0	0	++
				STERCULIACEAE							
3906	*Waltheria americana*	Guilca, Lima, Peru	4/54	l, s	−	...	.....	++	0	+	++
				THEACEAE							
3590	*Gordonia lasianthus*	Southport, Brunswick Co., N. C.	2/54	l, s	−	...	.....	++	0	+++	++
				ULMACEAE							
3005	*Holoptelea integrifolia*	C. G. F.	8/52	l	−	...	.....	0	0	0	0
				UMBELLIFERAE							
3771	*Chaerophyllum teinturieri*	Bullock Co., Ga.	4/54	l	−	...	.....	++	0	0	++

3808	*Conium maculatum*	Athens, Ga.	4/54 r, s	−	...	.....	0	0	0	++
3804	*Daucus carota*	Athens, Ga.	4/54 l	−	...	.....	+	0	0	++
3921	*Foeniculum vulgare*	Southport, Brunswick Co., N. C.	4/54 w	−	...	.....	0	0	0	+
3811	*Foeniculum vulgare*	Athens, Ga.	4/54 l, s, r	−	...	.....	0	0	0	+
3785	*Hydrocotyle bonariensis*	McIntosh Co., Ga.	4/54 l, s	−	...	.....	+	0	0	+
			URTICACEAE							
3946	*Boehmeria sp.*	Huallaga Riv., Tingo Maria, Peru	4/54 l, s	−	...	.....	0	0	0	++
3958	*Boehmeria sp.*	Rio Monzon, Tingo Maria, Peru	4/54 l, s	−	...	.....	0	0	++	++
3971	*Boehmeria sp.*	Bet. Puente Prado and Las Palmas, Tingo Maria, Peru	4/54 l, s	−	...	.....	0	0	0	+
3983	*Boehmeria sp.*	Huallaga Riv., Tingo Maria, Peru	4/54 l, s, b	−	...	.....	0	0	0	+++
			VALERIANACEAE							
3973	*Valeriana sp.*	Bet. Puente Prado and Las Palmas, Tingo Maria, Peru	4/54 l, s	−	...	.....	0	0	0	++
3810	*Valerianella radiata*	Athens, Ga.	4/54 l	−	...	.....	0	0	0	+
			VERBENACEAE							
3865A	*Aegiphila sp.*	W. of Tingo Maria, Peru	4/54 s, l	−	...	.....	0	0	+	+++
3865B	*Aegiphila sp.*	W. of Tingo Maria, Peru	4/54 b	−	...	.....	0	0	+	+
3889	*Aegiphila sp.*	E. of Divisoria, Tingo Maria, Peru	4/54 l, s, b	−	...	.....	0	0	0	++
3993	*Lantana sp.*	Rd. to Puento Nuevo, Tingo Maria, Peru	4/54 l, s	−	...	.....	0	0	0	+
3908	*Lantana sp.*	Quilca, Lima, Peru	4/54 l, s	−	...	.....	+	0	0	+
3773	*Verbena canadensis*	Emanual Co., Ga.	4/54 l, s	+	0	.....	++	0	0	++
3800	*Verbena tenuisecta*	Tattnall Co., Ga.	4/54 l, s	+	0	.....	+	0	0	+++
3879	*Verbena sp.*	Tingo Maria, Peru	4/54 l, s, r	−	...	.....	0	0	0	++
3037	*Vitex agnus-castus*	C. G. F.	9/52 l	−	...	.....	++	0	0	++
			VIOLACEAE							
3934	*Viola rafinesquii*	Athens, Ga.	4/54 l, s	−	...	.....	++	0	0	++
			VITACEAE							
3861	*Cissus sp.*	Rd. to Puento Nuevo, Tingo Maria, Peru	4/54 l, s	−	...	.....	0	0	0	+
			ZINGIBERACEAE							
3962	*Costus cylindricus*	Rio Manzon, Tingo Maria, Peru	4/54 l, s, b	−	...	.....	0	0	0	+++
			ZYGOPHYLLACEAE							
3483	*Guaiacum officinalis*	Matanzas, Cuba	5/53 l, b	+	0.2 mi	.....	0	0	0	+

[a] Bagasse from "heads" of agaves used for distilling mescal. [b] Yield may be high because of incorrect moisture.

CORRECTIONS ON FIRST 1,000 ACCESSIONS (2)

Page	Acces. No.	Should Read
1	909	*Agave collina*
1	956	*Agave pruinosa*
1	616	*Agave aff. schidigera*
3	337	*Agave mapisaga*
3	331	*Agave cantala*
3	959	*Agave mescal*
3	968	*Agave pacifica*
3	259	*Agave vilmoriniana*
3	603	*Agave vilmoriniana*
3	606	*Agave vilmoriniana*
3	608	*Agave vilmoriniana*
3	610	*Agave vilmoriniana*
4	973	*Agave flexispina*
4	977	*Agave flexispina*
4	963	*Agave patonii*
4	969	*Agave quiotifera*
6	865	*Agave augustifolia*
6	438	*Agave angustifolia sargentii*
6	860	*Agave collina*
6	458	*Agave aff. crassispina*
6	416	*Agave cupreata*
6	425	*Agave aff. lecheguilla*
6	448	*Agave lecheguilla*
6	441	*Agave mapisaga*
6	853	*Agave marmorata*
6	453	*Agave mitraeformis*
6	457	*Agave mitraeformis*
6	937	*Agave pacifica*
6	419	*Agave roezliana*
6	556	*Agave subsimplex*
6	864	*Agave verschaffeltii*
6	867	*Agave verschaffeltii*
6	618	*Agave yaquiana*
7	461	*Agave angustifolia*
7	519	*Agave americana*
7	734	*Agave collina*
7	731	*Agave aff. cupreata*
7	736	*Agave collina*
7	740	*Agave aff. cupreata*
7	907	*Agave aff. cupreata*
7	735	*Agave fenzliana*
7	922	*Agave geminiflora*
7	926	*Agave geminiflora atricha*
7	516	*Agave lecheguilla*
7	529	*Agave lophantha*
7	518	*Agave lophantha*
7	533	*Agave lophantha*
7	534	*Agave lophantha*
7	729	*Agave aff. lophantha*
24	931	*Yucca jaliscensis*
24	778	*Yucca peninsularis*
24	773	*Yucca peninsularis*
24	783	*Yucca peninsularis*

Page	Acces. No.	Should Read
7	739	*Agave mescal*
7	936	*Agave pacifica*
7	517	*Agave polyacantha*
7	521	*Agave polyacantha*
7	737	*Agave rubescens*
7	910	*Agave schidigera*
7	515	*Agave striata*
7	523	*Agave striata*
7	530	*Agave striata*
7	730	*Agave tequilana*
7	732	*Agave tequilana*
7	733	*Agave tequilana*
7	738	*Agave xalapensis*
8	432	*Agave cupreata*
8	443	*Agave cupreata*
8	428	*Agave kirchneriana*
8	972	*Agave aff. scabra*
8	985	*Agave aff. scabra*
11	929	*Sprekelia formosissima*
12	436	*Bromelia pinguin*
12	456	*Hechtia cf. podantha*
17	449	*Beaucarnea purpusii*
17	98	*Dasylirion texanum*
17	200	*Dasylirion wheeleri*
18	430	*Dasylirion acrotriche*
18	439	*Dasylirion acrotriche*
18	531	*Dasylirion acrotriche*
18	434	*Dasylirion lucidum*
18	971	*Dasylirion wheeleri*
18	954	*Nolina durangensis*
18	431	*Nolina nelsonii*
19	976	*Nolina juncea*
19	961	*Nolina juncea*
19	447	*Nolina nelsonii*
19	97	*Nolina texana*
19	499	*Ruscus hypoglossum*
21	203	*Yucca arizonica x Y. baccata*
		Yucca arizonica x Y. baccata
21	149	*Yucca baccata vespertina*
21	156	*Yucca baccata vespertina*
21	746	*Yucca sp.*
22	975	*Yucca macrocarpa*
23	87	*Yucca arkansana*
23	970	*Yucca rigida*
23	120	*Yucca torreyi*
24	450	*Yucca australis*
24	528	*Yucca australis*
24	525	*Hesperaloe funifera*
24	957	*Yucca decipiens*
24	459	*Yucca periculosa*
24	514	*Yucca sp.*
24	89	*Yucca treculeana*

CORRECTIONS ON SECOND 1,000 ACCESSIONS (4)

Page	Acces. No.	Should Read
2	1269	*Agave mescal, var.*
2	1795	*Agave aff. aurea*
2	1800	*Agave aff. aurea*
2	1825	*Agave aurea*
4	1799	*Agave aff. americana*
4	1818	*Agave fourcroydes*
4	1796	*Agave roseana*
5	1869	*Agave roseana*
5	1883	*Agave sobria*
5	1046	*Manfreda oliverana*
6	1283	*Thevetia ovata*
10	1273	*Amoreuxia palmatifida*
10	1281	*Amoreuxia palmatifida*
10	1289	*Amoreuxia palmatifida*

Page	Acces. No.	Should Read
12	1286	*Apodanthera palmeri*
12	1336	*Apodanthera palmeri*
12	1889	*Maximowiczia sonorae peninsularis*
16	1538	*Cnidoscolus aconitifolius*
16	1285	*Cnidoscolus augustidens*
16	1290	*Cnidoscolus multilobus* (probably)
16	1539	*Cnidoscolus multilobus* (probably)
16	1103	*Cnidoscolus tepicensis*
16	1547	*Cnidoscolus tubulosus* (probably)
16	1051	*Cnidoscolus urens*
16	1546	*Cnidoscolus urens*
21	1030	*Smilax spinosa*
22	1866	*Yucca valida, var.*
23	1288	*Martynia arenaria*

On page 5, at end of *Amaryllidaceae*, add: 1096 *Polianthes geminiflora*, Autlan, Jal., Mex., 8/51, bu, +, 0.3 mi.
On page 12, at end of *Convolvulaceae*, add: 1013 *Ipomoea sp.*, Nay., Mex., 7/51, t, +, 0, 0, 0, 0, 0. 1017 *Ipomoea sp.*, Nay., Mex., 7/51, t, +, 0, 0, 0, 0, +.

CORRECTIONS ON THIRD 1,000 ACCESSIONS (6)

Page	Acces. no.	Should Read
1	2373	*Agave pruinosa*
3	2330	*Agave stricta*
4	2328	*Agave lechuguilla, var.*
4	2201	*Agave tequilana*
4	2101	*Agave yaquiana*
4	2102	*Apave yaquiana*
4	2205	*Agave yaquiana*
4	2206	*Agave yaquiana*
4	2207	*Agave yaquiana*
5	2337	*Agave filifera*
6	2371	*Agave atrovirens, var.*
6	2325	*Agave aff. cochlearis*
6	2156	*Agave rhodocantha*
6	2338	*Agave aff. victorae-Regina*
6	2339	*Agave aff. victorae-Regina*
20	2621	*Allium haematochiton*
21	2512	*Yucca arizonica*

TABLE II.—OCCURRENCE OF THE VARIOUS SAPOGENINS BY SPECIES

Species	No. of Samples	Genin Content —M. F. B., %— Min.	Max.
CHLOROGENIN			
Agave marmorata	1	..	0.1
Agave schottii	1	..	0.4
Agave spp.	1	..	Tr.
Manfreda spp.	2	0.4	0.6
CORRELLOGENIN			
Dioscorea spiculiflora	2	..	0.2
Dioscorea sp.	1	..	0.2
9-DEHYDROHECOGENIN			
Agave aurea	3	..	Tr.
Agave brandegeei	2	..	Tr.
Agave cerulata	3	..	Tr.
Agave goldmaniana	2	..	Tr.
Agave mapisaga	1	..	Tr.
Agave mitraeformis	1	..	Tr.
Agave nelsonii	1	..	Tr.
Agave promontorii	5	..	Tr.
Agave roseana	1	..	Tr.
Agave sobria	1	..	Tr.
Agave vexans	1	..	Tr.
Manfreda sp.	1	..	Tr.
9-DEHYDROMANOGENIN			
Agave aurea	2	..	Tr.
Agave aurea x sobria	1	..	Tr.
Agave brandegeei	3	..	Tr.
Agave cerulata	7	..	Tr.
Agave desertii	1	..	Tr.
Agave goldmaniana	3	..	Tr.
Agave mirabilis	1	..	Tr.
Agave nelsonii	7	..	Tr.
Agave promontorii	1	..	Tr.
Agave scabra	1	..	0.4
Agave sullivanii	1	..	Tr.
Agave toumeyana	2	0.1	0.2
Agave vexans	1	..	Tr.
Agave spp.	3	..	Tr.
DIOSGENIN			
Agave angustiarum	1	..	0.2
Manfreda spp.	2	..	Tr.
Dioscorea bartlettii	2	0.1	0.4
Dioscorea composita	10	Tr.	10.1
Dioscorea macrostachya	3	0.1	1.5
Dioscorea nelsonii	3	Tr.	1.8
Dioscorea platycolpota	1	..	0.4

TABLE II.—*Contd.*

Species	No. of Samples	Genin Content —M. F. B., %— Min.	Max.
DIOSGENIN			
Dioscorea polygonoides	1	..	0.3
Dioscorea spiculiflora	2	0.7	1.5
Dioscorea sylvatica	1	..	2.4
Dioscorea tepinapensis	3	0.4	0.7
Dioscorea spp.	55	0.4	3.7
GENTROGENIN			
Dioscorea spiculiflora	2	..	0.2
Dioscorea sp.	1	..	0.2
GITOGENIN			
Agave brandegeei	1	..	Tr.
Agave cerulata	2	..	Tr.
Agave desertii	1	..	Tr.
Agave fourcroydes	1	..	Tr.
Agave mirabilis	1	..	Tr.
Agave mitraeformis	1	..	Tr.
Agave promontorii	1	..	Tr.
Agave roseana	2	..	Tr.
Agave aff. schidigera	1	..	Tr.
Agave sobria	1	..	Tr.
Agave toumeyana	2	..	Tr.
Agave vilmoriniana	1	..	0.4
Agave spp.	3	Tr.	0.8
Furcraea cabuya	1	..	0.1
Furcraea spp.	3	..	Tr.
Manfreda sp.	7	0.1	0.7
Yucca peninsularis	4	0.2	0.6
Yucca whipplei	1	..	0.3
HECOGENIN			
Agave angustifolia	1	..	0.7
Agave applanata	1	..	Tr.
Agave aurea	4	0.05	0.7
Agave aurea x sobria	1	..	0.4
Agave brandegeei	5	0.3	0.5
Agave cerulata	9	Tr.	1.7
Agave desertii	1	..	0.3
Agave deweyana	1	..	Tr.
Agave ferox	1	..	0.2
Agave goldmaniana	3	Tr.	0.5
Agave letonae	1	..	Tr.
Agave lophantha	1	..	Tr.
Agave mapisaga	1	..	Tr.
Agave mirabilis	1	..	0.4
Agave mitraeformis	1	..	0.2
Agave nelsonii	5	Tr.	0.7
Agave pampaniniana	1	..	0.4
Agave promontorii	9	0.1	1.8
Agave roseana	4	Tr.	1.0
Agave aff. schidigera	1	..	Tr.
Agave sobria	5	0.1	0.3
Agave sullivanii	1	..	0.5
Agave toumeyana	2	0.4	0.5
Agave verschaffeltii	1	..	Tr.
Agave vexans	2	0.2	0.4
Agave spp.	6	Tr.	1.5
Furcraea spp.	6	Tr.	0.2
Manfreda sp.	1	..	Tr.
Yucca peninsularis	3	0.1	0.5
KAMMOGENIN			
Yucca filamentosa	1	..	0.3
MANOGENIN			
Agave applanata	1	..	Tr.
Agave aurea	2	..	Tr.
Agave aurea x sobria	1	..	Tr.
Agave brandegeei	3	..	Tr.
Agave cerulata	9	Tr.	0.5
Agave desertii	1	..	Tr.
Agave deweyana	1	..	Tr.

Contd. page 684

TABLE II.—*Contd.*

Species	No. of Samples	Genin Content —M. F. B., %— Min.	Max.
MANOGENIN			
Agave ferox	1	..	Tr.
Agave fourcroydes	1	..	Tr.
Agave funkiana	1	..	Tr.
Agave goldmaniana	3	Tr.	0.4
Agave mirabilis	1	..	Tr.
Agave nelsonii	7	Tr.	1.0
Agave promontorii	7	..	Tr.
Agave roseana	1	..	Tr.
Agave scabra	1	..	0.4
Agave aff. schidigera	1	..	Tr.
Agave sobria	4	Tr.	0.2
Agave sullivanii	1	..	0.7
Agave toumeyana	2	0.3	0.4
Agave vexans	2	..	Tr.
Agave spp.	3	..	Tr.
Furcraea cabuya	1	..	0.1
Furcraea spp.	3	..	Tr.
Manfreda sp.	1	..	0.4
MARKOGENIN			
Yucca schidigera	1	..	0.4
SARSASAPOGENIN			
Agave sp.	1	..	0.8
Yucca schidigera	4	0.5	1.3
SMILAGENIN			
Agave cf. funkiana	1	..	0.5
Agave marmorata	2	0.05	0.6
Agave vilmoriniana	3	0.8	3.2
TIGOGENIN			
Agave applanata	1	..	Tr.
Agave aurea	1	..	Tr.
Agave aurea x sobria	1	..	Tr.
Agave brandegeei	3	Tr.	1.8
Agave cerulata	4	..	Tr.
Agave deweyana	1	..	0.05
Agave ferox	1	..	Tr.
Agave fourcroydes	1	..	0.2
Agave funkiana	1	..	0.3
Agave goldmaniana	1	..	Tr.
Agave letonae	1	..	0.1
Agave lophantha	1	..	0.2
Agave mapisaga	1	..	Tr.
Agave mirabilis	1	..	Tr.
Agave nelsonii	5	Tr.	1.2
Agave promontorii	7	Tr.	0.5
Agave roseana	4	Tr.	0.9
Agave schottii	1	..	Tr.
Agave sobria	3	..	Tr.
Agave sullivanii	1	..	Tr.
Agave toumeyana	1	..	0.1
Agave verschaffeltii	3	0.2	0.6
Agave spp.	6	0.1	0.4
Furcraea cabuya	1	..	0.2
Furcraea spp.	6	0.2	0.7
Manfreda spp.	3	0.3	0.4
Yucca peninsularis	6	0.2	2.0
Yucca whipplei	3	0.7	0.9
Yucca sp.	1	..	0.9
YAMOGENIN			
Agave cf. funkiana	1	..	0.1
Agave vilmoriniana	1	..	0.4
Dioscorea bartlettii	3	0.3	0.4
Dioscorea composita	1	..	0.5
Dioscorea macrostachya	1	..	0.05
Dioscorea spiculiflora	2	0.4	0.9
Dioscorea spp.	13	0.5	2.1

Practically all *Agave*, *Dioscorea*, and *Yucca* contained saponins but the proportion of steroidal sapogenins was, respectively, 60, 34, and 80%.

Two new steroidal sapogenins are reported in this list. They are the isomeric gentrogenin and correllogenin, from two lots of *D. spiculiflora* and from one unidentified *Dioscorea*, all from Chiapas, Mexico. A preliminary description of their chemistry has been published (7). They always seem to occur with diosgenin and yamogenin. Two other new steroidal sapogenins have recently been found: ruscogenin from *Ruscus aculeatus* (8) and rhodeasapogenin from *Rhodea japonica* (9).

A few new records of high content of sapogenins are reported: 1.85% hecogenin in *Agave promontorii*, 1.0% manogenin in *A. nelsonii*, 3.2% smilagenin in *A. vilmoriniana*, and 2.1% yamogenin in a *Dioscorea sp.*

For the first time in this series the sapogenins of *Furcraea* could be identified. Tigogenin, gitogenin, manogenin, and hecogenin were found—the highest total being 1.3%.

Steroidal sapogenins are reported in 10 new species of *Dioscorea* and in 20 new species of *Agave*.

Flavonoids were found in 48 species, of which 42 are new to the record.

Alkaloids were found in eight species, of which the following are new to the record: *Urechites lutea* (Apocynaceae); *Albizzia polyphylla*, *Lupinus diffusus*, *L. villosus* (Leguminosae).

Tannins were absent in the 53 species of Compositae so far examined. In the other families in the present list there are too few collections for generalizations to be drawn.

Unsaturated sterols continued to be of common occurrence.

Ficus is not a promising genus for the group of constituents sought in this series. Fifty-nine species have yielded practically nothing but sterols. Similarly, *Agave*, *Yucca*, and *Dioscorea* have given so few positive tests for all four nonsteroid groups that in the present series we have not made the tests.

REFERENCES

(1) Wall, M. E., Krider, M. M., Krewson, C. F., Eddy C. R., Willaman, J. J., Correll, D. S., and Gentry, H. S., THIS JOURNAL, **43,** 1(1954).
(2) Wall, M. E., Krider, M. M., Krewson, C. F., Eddy, C. R., Willaman, J. J., Correll, D. S., and Gentry, H. S., U. S. Dept. Agr., Agr. Research Service Circ. AIC-363, 25, 1954.
(3) Wall, M. E., Eddy, C. R., Willaman, J. J., Correll, D. S., Schubert, B. G., and Gentry, H. S., THIS JOURNAL, **43,** 503(1954).
(4) Wall, M. E., Eddy, C. R., Willaman, J. J., Correll, D. S., Schubert, B. G., and Gentry, H. S., U. S. Dept. Agr., Agr. Research Service Circ. AIC-367, 32, 1954.
(5) Wall, M. E., Fenske, C. S., Willaman, J. J., Correll, D. S., Schubert, B. G., and Gentry, H. S., THIS JOURNAL, **44,** 438(1955).
(6) Wall, M. E., Fenske, C. S., Willaman, J. J., Correll, D. S., Schubert, B. G., and Gentry, H. S., U. S. Dept. Agr., Agr. Research Service Circ. ARS-73-4, 30, 1955.
(7) Walens, H. A., Serota, S., and Wall, M. E., *J. Am. Chem. Soc.*, **77,** 5196(1955).
(8) Lapin, H. and Sannié, C., *Bull. soc. chim. France*, 1552(1955).
(9) Nawa, H., *Proc. Japan Acad.*, **29,** 214(1953).
(10) Wall, M. E., Krider, M. M., Rothman, E. S., and Eddy, C. R., *J. Biol. Chem.*, **198,** 533(1952).
(11) Wall, M. E., Eddy, C. R., McClennan, M. L., and Klumpp, M. E., *Anal. Chem.*, **24,** 1337(1952).
(12) Eddy, C. R., Wall, M. E., and Scott, M. K., *ibid.*, **25,** 266(1953).

A Potent Pyrogenic Extract Derived from a Strain of *Aerobacter aerogenes**

By R. J. REEDY, E. J. OSWALD, and J. K. NIELSEN

Utilizing a simple technic, a good yield of pyrogenic material has been obtained from a strain of *Aerobacter aerogenes*. This dried product consistently elicits pyrogenic response in rabbits in dilutions of approximately 1:100,000,000 (0.01 μg./ml.) and is relatively stable to dry heat when exposed to 120° for forty minutes.

LITERALLY HUNDREDS of pyrogenic preparations have been described in the literature in the years that have intervened since Hort and Penfold (1), and Seibert (2) first recognized and investigated these often troublesome substances more than three decades ago. Since then much has been learned of their physical and chemical properties which, though varying to some degree in the individual materials isolated, have shown many characteristics in common. Moreover, information has accumulated recently which indicates that they have a possible place as a chemotherapeutic adjunct (3-6). In this connection, as well as in the interest of learning more of the basic mode of action of such a substance in the animal body, the present work has been undertaken.

By means of a simple method an excellent yield of a high potency pyrogen has been obtained. It is heat stable, and varies little in the two separate batches prepared as to hygroscopicity and certain other physical properties. Its preparation, potency, and heat stability will be discussed in this preliminary report.

MATERIALS AND METHODS

The extraction method employed is essentially that suggested by Fischbach and Levine (7) of the Food and Drug Administration. A strain of *Aerobacter aerogenes* (#114) isolated from a commercial streptomycin sample was the organism used. Carboys of 20-liter capacity, provided with spargers and sterile air intakes and containing Koser's[1] medium were seeded with this organism. At a constant temperature of 37° with minimum airflow agitation, growth was allowed to proceed for three to four days. At the end of this period, the entire contents was subjected to flash evaporation and reduced to one-fifth of the original volume. Four volumes of acetone were added to the concentrate and mixed with a mechanical stirrer for one-half hour. The precipitate was allowed to settle overnight at room temperature and the supernatent fluid decanted and discarded. A small amount of water was added to the precipitate to form a slurry, and this mixture was poured into a side-arm suction flask. At a temperature of 37° under reduced pressure, all of the residual acetone was boiled off. The mucoid material which separated out at this point was kept apart for future testing. The liquid portion was placed in the refrigerator overnight, and all formed crystals discarded. The remaining liquid was passed through a 3.5 x 120-cm. ion exchange column containing Amberlite® (MB-3) which had been suspended in distilled water and the water removed. The rate of flow of liquid through the column was approximately 5 ml. per minute. The material was allowed to flow until the column was three-quarters exhausted. The column was then emptied, refilled with Amberlite, and the remaining liquid passed through. The final pH of the liquid so treated was approximately 5.0. This was lyophilized and the final, dried product subjected to grinding, using either a ball mill or mortar and pestle. The approximate yield of powder thus prepared was 5.0 Gm. from 240 liters of Koser's citrate broth.

A multiple dose method, proposed by one of us (RJR), utilizing single rabbits was employed in the determination of pyrogen titers. Beginning with the least amount of material in solution in any single test, four intravenous injections of increasing concentration were made at two-hour intervals. Prior to the initial injection, a control temperature was taken and subsequent temperatures were measured at hourly intervals. The highest rise within each two-hour interval, compared to the control temperature, was used to determine the pyrogen titer. Dose response in rabbits so treated was found to be reproducible within acceptable limits.

RESULTS AND DISCUSSION

Two batches of material were prepared and compared to a material presently used by this laboratory as a pyrogen "House Standard." The latter was obtained by extraction from a strain of *Pseudomonas aeruginosa* in the manner here described. Each batch was tested in rabbits. The multiple dose method was checked in two instances by the injection of single levels in several rabbits with good comparative results. Table I shows the temperature rise obtained in rabbits injected with the indicated amounts of material.

In order to determine the extent of any cumulative effect of this titration method in rabbits, subpyrogenic doses (on the basis of previously determined pyrogenic titer) were injected into 10 rabbits

* Received March 21, 1957, from the Division of Antibiotics; Food and Drug Administration; Department of Health, Education, and Welfare; Washington, D. C.

[1] A simple Koser's Citrate Medium (dehydrated) was used to avoid extraneous protein material (Difco Laboratories, Inc., Detroit, Mich.).

TABLE I.—POTENCY MULTIPLE DOSE METHOD. DEGREES TEMPERATURE RISE OBTAINED IN INJECTED RABBITS

	Hrs. after Control Temperature 2	4	6	8
	μg./ml.			
	0.005	0.01	0.02	0.04
Batch 1	0.3	0.7	1.1	1.5
	0.4	0.5	1.1	1.7
	0.4	0.9	0.9	1.6
Av.[a]	0.36	0.70	1.03	1.60
Batch 2	0.5	1.0	1.6	1.7
	1.5	2.5	2.7	2.8
	0.9	1.5	2.2	2.6
Av.[a]	0.96	1.66	2.17	2.37
	0.04	0.1	0.2	1.0
Present "House Standard"	0.2	0.5	0.8	1.4
	0.3	0.6	0.6	1.1
Av.[a]	0.25	0.55	0.70	1.25

[a] The averages given for Batch 1 represent the multiple dose assay results obtained in three rabbits. Three rabbits were used to obtain the average figures for Batch 2. Two rabbits were used in obtaining the averages presented for the "house standard" material.

TABLE II.—HEAT STABILITY. DEGREES TEMPERATURE RISE ELICITED IN RABBITS BY MATERIAL HEATED FOR FORTY MINUTES AT 120°

	Hrs. after Control Temperature 2	4	6	8
	μg./ml.			
	0.01	0.02	0.04	0.1
Batch				
1[a]	0.4	0.6	0.7	1.3
2[a]	0.2	0.4	0.7	0.9
	0.2	0.4	1.0	2.0
Present "House Standard"[a]	0.2	0.3	0.3	0.5
	0.1	0.0	0.2	0.4
Av.	0.15	0.15	0.25	0.45

[a] In single rabbits Batches 1 and 2 gave the indicated temperature rises above the control temperatures. The "house standard" material was tested in duplicate.

TABLE III.—ELEMENTAL CHEMICAL ANALYSIS (PER CENT) OF THREE PYROGEN PREPARATIONS

	Batch 1	Batch 2	"House Standard" Pyrogen
Moisture	7.75	7.57	5.52
Nitrogen	8.74	6.80	9.06
Hydrogen	7.58	7.64	6.94
Carbon	47.72	46.56	46.60
Sulfur	0.78	0.49	0.69
Phosphorus	0.85	0.94	3.74

using the multiple dose technique. Of 40 temperature observations made, nine gave rises of 0.6° or more, eight of the nine in the sixth and eighth hour. The same rabbits were retested after a two-week rest period. Of 40 temperature observations made on these, rises of 0.6° or more were observed seven times, all in the sixth- and eighth-hour period. It may be said that there is some indication of a cumulative pyrogenic effect using this method, although this in no way invalidates the responses observed in the *greatest dilution* utilized in the test, since here no prior injection had been made. As far as pyrogen tolerance is concerned, it is not thought, on the basis of present knowledge, that this can be built up in a matter of hours. Days at least are required before resistance to these materials appears in rabbits.

In order to determine the heat stability of these materials, weighed aliquots were heated in an oven for forty minutes at 120°. Table II compares the pyrogenic rise obtained in different batches under these conditions.

It can be seen from these results that activity remained in both batches after heating, although it was necessary to increase injected amounts from two- to eight-fold in order to elicit a pyrogenic response. This indicates a relatively heat-stable product when compared to the present pyrogen "house standard," which, when increased ten-fold in concentration failed to give temperature rises in rabbits, under similar conditions. The batches of *A. aerogenes* pyrogen were much alike in their initial high activity in rabbits and heat stability under the conditions imposed, and differed only slightly in their physical properties. Table III presents their elemental chemical analysis and moisture content.

Toxicity tests performed intravenously in 19–21 Gm. mice gave an LD^0 of 500 μg./mouse and an LD^{100} of 5000 μg./mouse in forty-eight hours. The material has not been tested for antigenicity.

The activity of these pyrogen preparations represents a 20- to 40-fold increase in activity over the *Pseudomonas* preparation presently in use as a "house standard" and compares favorably with the most active preparations reported in the literature thus far (8–10).

REFERENCES

(1) Hort, E. C., and Penfold, W. J., *Proc. Roy. Soc. Med.*, **5**, 131(1912).
(2) Seibert, F. B., *American J. Physiol.*, **67**, 90(1923).
(3) Favez, G., Agnet, A., and Bossy, A., *Helv. Med. Acta*, **21**, 464(1954).
(4) Chesrow, E. J., Piszczek, E., Garner, H. H., and Kaplitz, S., *Geriatrics*, **8**, 504(1953).
(5) Brues, A. M., and Shear, J. J., *J. Natl. Cancer Inst.*, **5**, 195(1944).
(6) Olsen, W. H., and Necheles, H., *Am. J. Digest Diseases*, **20**, 372(1953).
(7) Fischbach, H., and Levine, J. (to be published).
(8) McGinty, D. A., Wilson, M. L., and Rodney, G., *Proc. Soc. Exptl. Biol. Med.*, **70**, 334(1949).
(9) Beck, L. W., and Fisher, M., *Cancer Research*, **6**, 410(1946).
(10) Eichenberger, Van E., *et al.*, *Schweiz. med. Woch.*, **85**, 1213(1955).

A Rapid Chromatographic Assay of Acetylsalicylic Acid, Acetophenetidin, and Caffeine*

By JOSEPH LEVINE

A rapid procedure for the total analysis of acetylsalicylic acid, acetophenetidin, and caffeine by a modified partition chromatographic technique is presented. Acetophenetidin and caffeine are separated in a manner similar to previous chromatographic separations. Acetylsalicylic acid and any salicylic acid formed by hydrolysis are trapped on a segment of the column in which sodium bicarbonate is the immobile phase; these acids are released and eluted by a chloroform solution of acetic acid. The concentrations of each substance are measured spectrophotometrically. The complete analysis may be carried out in one hour.

A NOTABLE IMPROVEMENT in the determination of acetylsalicylic acid, caffeine, and acetophenetidin (APC) was achieved by Higuchi and Patel (1), who applied partition chromatography to the previously lengthy separation of the latter two components. After an initial separation of acetylsalicylic acid from the mixture by conventional shake-out techniques, they chromatograph the two-component mixture over a silicic acid-water partition column. Isopropyl ether is used to elute the acetophenetidin, and chloroform to elute the caffeine.

Modifications of this procedure were introduced by Banes (2) and by Smith (3), who substituted Celite® for silicic acid as supporting phase. Banes (2) used ethyl ether in place of isopropyl ether for the acetophenetidin elution.

In the procedure herein described, chromatographic separation of all three components is achieved without any preliminary shake-out. Partition is effected over a duplex column with 4 *N* sulfuric acid being used as stationary phase on the lower segment of the column, and 1 *N* sodium bicarbonate on the upper segment. Celite is used as supporting phase. Smaller quantities of adsorbent are used than in the previous procedures; 2 Gm. of Celite and 2 ml. of immobile phase are used in each segment. Use of the smaller amounts simplifies the preparation of the column and permits a very rapid flow of eluate.

The very strong acid solution is used to effect complete entrapment of caffeine during the elution of acetophenetidin. Using water as stationary phase, caffeine is incompletely trapped. Slight leakage results even when 2 *N* hydrochloric acid is used, as in Banes' procedure (2). With the 4 *N* sulfuric acid, however, no trace of caffeine is removed by the solvent used to elute the acetophenetidin. The caffeine is readily eluted from the acid, after removal of the acetophenetidin, by chloroform.

The sodium bicarbonate phase traps both acetylsalicylic acid and any salicylic acid formed by hydrolysis in the tablets. The acidic material is recovered, subsequent to the removal of acetophenetidin and caffeine, by elution with a solution of acetic acid in chloroform which neutralizes the sodium bicarbonate constituting the immobile phase and liberates the free acids. No hydrolysis of acetylsalicylic acid occurs during the separation.

Measurements of the isolated components are made spectrophotometrically. The eluates containing caffeine and acetylsalicylic acid are collected in volumetric flasks and read directly; the acetophenetidin eluate is evaporated and the residue dissolved in a chloroform-isooctane mixture to a concentration suitable for measurement. The absorption maxima of both acetylsalicylic acid and salicylic acid in chloroform shift to higher wavelengths and increase in intensity when acidified; this parallels the increase in aqueous solution upon acidification. Hence, a sufficient excess of acetic acid is used in the eluent to provide an adequate concentration in the eluate.

The sample is prepared for introduction onto the column by placing a weighed portion of powdered tablet into a volumetric flask, mixing thoroughly with chloroform containing a small amount of acetic acid, and adjusting to volume. Without separation of insoluble excipient material, an aliquot is diluted with ether and passed directly onto the column. The acetic acid is added to convert any acetylsalicylic acid salt which may be present to the free acid.

The procedure is very rapid. Solvent flows through the column at a rate approximating 5 to 10 ml. per minute. The entire chromatographic separation, from the time of introduction of the

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TABLE I

Acetophenetidin Added, Mg.	Acetophenetidin Found, Mg.	Caffeine Added, Mg.	Caffeine Found, Mg.	Acetylsalicylic Acid Added, Mg.	Acetylsalicylic Acid Found, Mg.	Salicylic Acid Added, Mg.	Salicylic Acid Found, Mg.
2.50	2.45	0.50	0.49	5.00	4.95	0.00	0.004
2.50	2.45	0.50	0.49	5.00	5.06	0.00	0.00
2.50	2.46	0.50	0.49	4.00	3.97	0.37	0.43
2.50	2.53	0.50	0.50	5.00	5.01	0.76	0.82
2.50	2.51	0.50	0.49	0.00	0.00	1.89	1.88

TABLE II

	Acetophenetidin, Grains per Tablet			Caffeine, Grains per Tablet			Acetylsalicylic Acid, Grains per Tablet				
		Found			Found			Found			
Sample	Labeled	Present Assay	N. F. X Assay	Labeled	Present Assay	N. F. X Assay	Labeled	Intact	Present Assay Hydrolyzed	Total	N. F. X Assay
A	2.50	2.40	2.61	0.50	0.46	0.43	3.50	3.61	0.00	3.61	3.40
		2.42			0.49			3.60	0.00	3.60	
B	2.50	2.33	2.54	0.50	0.49	0.44	3.50	3.37	0.12	3.49	3.03
		2.40			0.50			3.33	0.13	3.46	
C[a]	2.00	2.08	2.24	0.25	0.25	0.25	3.00	2.43	0.53	2.96	2.98
		2.07			0.26			2.42	0.54	2.96	

[a] Sample *C* contains aluminum hydroxide and flavor.

sample until the passage of the final eluate, requires approximately one-half hour and an entire analysis, including the weighing of the sample and the spectrophotometric readings, can be made in about one hour.

PROCEDURE

Preparation of Sample.—Ascertain the average weight per tablet. Grind to a fine powder a sufficient number of tablets to provide a representative sample. Transfer a weighed sample containing approximately 50 mg. acetylsalicylic acid to a 50-ml. volumetric flask. Add 30 ml. U. S. P. chloroform and shake thoroughly. Add 0.1 ml. glacial acetic acid and make to volume with chloroform.

Chromatographic Column.—The column is made of a 25 x 200-ml. test tube to which is attached an approximately 5 cm. length of 6–8 mm. tubing. The tamping rod consists of a disk of stainless steel, aluminum, or glass, with a diameter 1 mm. less than that of the column, attached to a rod 12 to 18 inches long.

Pack fine glass wool in the base of the column to act as a support.

To 2.0 Gm. Celite 545[1] in a glass mortar add 2.0 ml. of approximately 4*N* H_2SO_4 (9 H_2O + 1 concd. H_2SO_4). Incorporate thoroughly by kneading with a flexible spatula blade. Transfer to column and tamp, using gentle pressure, to compress the material to a uniform mass. In like manner, mix 2.0 Gm. Celite with 2.0 ml. of approximately 1 *N* $NaHCO_3$ and place in column above the foregoing contents. Wash column with 15–20 ml. water-washed ether and discard washings.

Assay.—(Use water-washed solvents in the ensuing procedure.) Dilute 5.0 ml. of sample preparation with 20 ml. ether and pass over column. After last portion of this solution has passed onto the adsorbent, wash with five 5-ml. portions of ether, allowing each portion to pass into the adsorbent prior to the addition of the ensuing one. Rinse tip of outlet tube with chloroform and evaporate the total eluate to dryness on steam bath in a current of air. This fraction contains the acetophenetidin. Dissolve the residue in 5 ml. chloroform and make to volume with isooctane in a 50-ml. volumetric flask.

Immediately after passage of last portion of ether through the column replace receiver with a 50-ml. volumetric flask. Pass 48 ml. chloroform through column, rinse tip with chloroform, and dilute eluate to volume. This fraction contains the caffeine.

Replace receiver with 100-ml. volumetric flask. Pass a solution of 0.5 ml. glacial acetic acid in 5 ml. chloroform through the column followed by 90 to 92 ml. of a 1% solution of acetic acid in chloroform and dilute to volume. This fraction contains both acetylsalicylic and salicylic acid.

In a suitable spectrophotometer, read the absorbances of the solutions. Compare the values obtained with those determined simultaneously on solutions of the pure materials. Chloroform containing 1% acetic acid is used for the solutions of salicylic acid and acetylsalicylic acid. The latter standard must be prepared daily.

The absorbance of acetophenetidin at a concentration of 50 mcg. per ml. is about 0.505 at 285 mμ; of caffeine at 10 mcg. per ml., 0.485 at 276 mμ; of acetylsalicylic acid at 100 mcg. per ml., 0.790 at 280 mμ and 0.010 at 310 mμ; and of salicylic acid at a concentration of 25 mcg. per ml., calculated as acetylsalicylic acid, 0.113 at 280 mμ and 0.573 at 310 mμ.

For practical purposes, the concentrations of acetylsalicylic and salicylic acids in a mixture can be calculated by neglecting the absorbance of acetylsalicylic acid at 310 mμ and attributing the entire absorbance at this wavelength to salicylic acid.

[1] Johns-Manville Corp.

From this value, the absorbance due to salicylic acid at 280 mμ can be calculated, and deducted from the total absorbance at this wavelength; the remainder can be considered to be the absorbance of the acetylsalicylic acid.

For greater precision, a second approximation may be made: from the concentration of acetylsalicylic acid calculated above, the absorption at 310 mμ due to the salicylic acid can be calculated and deducted from the total absorption at 310 mμ. From the resultant value, a more exact value for the concentration of salicylic acid may be made, and, from this, a correspondingly more exact value for its absorption at 280 mμ, is calculated, thereby obtaining a truer value for the absorption due to acetylsalicylic acid at 280 mμ.

RESULTS

Mixtures of acetylsalicylic acid, acetophenetidin, caffeine, and salicylic acid were assayed by the above procedure. Recoveries are shown in Table I.

Three commercial samples of acetylsalicylic acid, acetophenetidin, and caffeine tablets were assayed by the present procedure and by the National Formulary X procedure. Sample *C*, containing aluminum hydroxide and flavor, formed intractable emulsions when the chloroform extract prepared according to the N. F. procedure was extracted immediately. Upon standing overnight, flocculent material separated in the extract; after filtration of this solution, extraction resulted in moderate emulsification. This sample offered no difficulty in the present procedure. Results of the analyses are presented in Table II.

DISCUSSION

The procedure herein described differs markedly from conventional partition chromatographic techniques in that it provides for the alteration of the nature of the immobile phase as a step in the process, while in the earlier techniques the constitution of immobile phase remains unchanged. The versatility of partition chromatography is thus broadened to permit separations not previously possible. Thus, in the separation described, the sodium bicarbonate in the immobile phase functions as a trap to immobilize acetylsalicylic acid while the other components of the mixture are removed. In the ensuing step, the immobile phase is changed via neutralization by acetic acid which is introduced as a component of the eluant; the immobile phase, which now consists of sodium acetate, releases the acetylsalicylic acid for elution by the eluant.

In samples in which acetylsalicylic acid has become partially hydrolyzed, both the intact and the hydrolyzed portions are determined individually.

Preliminary experiments now in progress show that in a like manner the converse process is feasible: a strongly acidic immobile phase may be used to trap alkaloidal compounds, which in turn are liberated by a solution of an aliphatic amine. These will be reported in a forthcoming publication.

REFERENCES

(1) Higuchi, T., and Patel, K. P., THIS JOURNAL, **41**, 171(1952).
(2) Banes, D., *ibid.*, **44**, 713(1955).
(3) Smith, G., *J. Assoc. Offic. Agr. Chemists*, **37**, 677(1954).

Triazine Mercurial Diuretics*

By SEYMOUR L. SHAPIRO, VINCENT A. PARRINO, and LOUIS FREEDMAN

A series of mercurated N-allylguanamines has been prepared and evaluated as mercurial diuretics. The experimental data reflect that compounds of varying diuretic activity are obtainable by appropriate group variation within I. Diuretic activity is also found in a series of tris-mercurials synthesized by mercuration of tri-allylcyanurate.

THE MANY YEARS of clinical experience with mercurial diuretics have established that such drugs used with proper precaution are remarkably effective (1). However, numerous instances of untoward side effects (2) associated with such therapy have been a deterrent to wider usage as well as a stimulus (3) to the medicinal chemist to prepare improved mercurials.

Since the discovery of mercurial diuretics by Vogl (4) in 1919, many compounds have been reported in which desirable improvements in stability (5), safety (6), tolerance (7), potency (8), solubility (9), oral effectiveness (3, 10), and combinations of these factors have been achieved.

With few exceptions the mercurial diuretics in clinical usage are derivable from mercuration of

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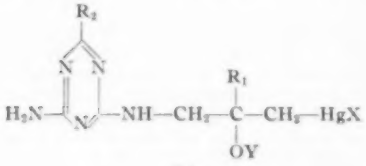
an allylamide structure of the type, D-NH-CH_2-CH(OY)-CH_2-HgX, wherein D is an organic nucleus affording a point of attachment for the mercurated allylamine linkage.

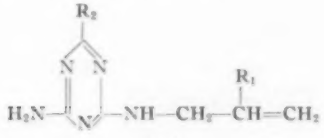
This investigation was concerned with the synthesis of new mercurials embodying the *s*-triazine ring in an attempt to obtain compounds effective as diuretics at low dosage levels. In particular, the nucleus D was varied to embody the formoguanamine nucleus, known to have diuretic activity *per se* (11) and the acetoguanamine nucleus which manifests only slight diuretic activity (12).

Typical of the structures prepared is I:



(I)

which was obtained by the following sequence of reactions: The allylamine hydrochloride (R_1 = H and CH_3) was fused with dicyandiamide to yield the biguanide (13) which in turn was cyclized with ethylformate (14) (R_2 = H) or acetyl chloride (15) (R_2 = CH_3) to the triazine II. In the case R_1 = H and R_1 = CH_3 cyclization to the triazine II, R_2 = H was effected without isolation of the biguanide. Treatment of II with mercuric acetate (8) in the solvent YOH (Y = H, CH_3, C_2H_5 and *n*-C_3H_7)



(II)

with probable mercuration at the terminal carbon atom (16) gave I isolable as I, X = OAc or OH. Conversion of I, X = OAc or OH to X = theophylline, carbonylsalicylamide (1,3,2-benzoxazine-2,4-dione),8-methylcarbonylsalicylamide, chloride, bromide, ascorbyl and potassium acid fluoride was effected in an aqueous system. The use of X = carbonylsalicylamide (17) evaluated a group, which in molecular size and character of mercurial bonding, was analogous to the theophylline linkage which has been reported to reduce toxicity (18) in certain diuretic structures.

Ascorbic acid has been reported to have a favorable effect on mercurial diuresis (19) and this suggested the use of X = ascorbyl. Finally, the inhibitory effect of fluoride ion on enzyme systems, coupled with the role of enzymes in the mode of action of mercurial diuretics (20, 21) indicated investigation of a mercury fluorine linkage.

DISCUSSION

The compounds prepared were evaluated parenterally in dogs and the data has been summarized in Table I.

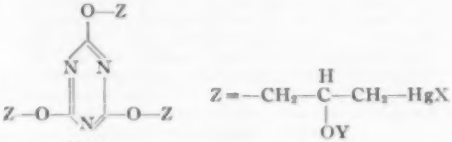
Certain structural relationships are admissible upon inspection of the pharmacological data. The compounds I, R_2 = H, have acute subcutaneous toxicities, probably about twice as great as those in mercurials now in clinical use (22) while I, R_2 = CH_3 compounds are probably more toxic. In general, variants of I, R_2 = H, show a more favorable diuretic response than I, R_2 = CH_3 (compounds 1 *vs.* 17; 2 *vs.* 18; 5 *vs.* 22; however, see 20 *vs.* 4).

Correlation of structure and activity indicates that Y = CH_3 is associated with the same favorable pattern (compounds 5 *vs.* 3, 11, 13; 21 *vs.* 18) that has been found by others (23, 24). The use of R_1 = CH_3 as compared to R_1 = H (compound 15 *vs.* 5) did not have the favorable effect on diuresis noted by Rowland *et al.* (8b), in their series. Variation of X yielded considerable differences in response in the group I, R_2 = H, X = theophylline, carbonylsalicylamide and chlorine were the most potent (compounds, 5, 6, 7 *vs.* 4, 8; 3, 2 *vs.* 1) while in the category I, R_2 = CH_3, X = OAc showed the best response (compounds 17 *vs.* 18, 19; 20 *vs.* 21, 22). The fluoride derivative (compound 8) was not as effective as some of its structural analogs (compounds 5, 6, 7), although it had a pronounced diuretic effect. The ascorbyl derivative (compound 9) was not tested.

While the findings with selected I (compound 5) in these animal experiments parallel the oral and intravenous diuretic activity of 3-chloromercuri-2-methoxypropyl urea, the most potent mercurial in clinical use, the hazards of translation of results of animal testing to human use (23, 25) are well recognized.

Diuretics containing two (26, 27) and three (27) mercury atoms per molecule have been prepared.

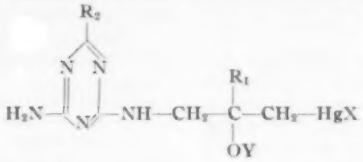
An additional phase of this work entailed the preparation of triazine polymercurials derivable from triallyl cyanurate to give structures of the type III, containing three mercury atoms per molecule:



(III)

In this series Y was varied as CH_3 and C_2H_5, and the compounds isolated as III, X = OH which was converted to III, X = theophylline. The diuretic response in rats obtained with III, Y = CH_3, and X = theophylline, was considerably better than that obtained with a comparable dosage level of mercurophylline.

TABLE I.—MERCURATED ALLYL TRIAZINES



Compound No.	Y	X	M. p.,[a] °C.	Formula	Nitrogen, %[b] Calcd.	Found	LD min.[c]	A[d] (S. E.)
R_1, R_2 = H								
1	H	OAc	127	$C_8H_{12}HgN_5O_2$	16.3	16.0	50	79 (1.4)
2	H	Cl	191	$C_6H_{10}ClHgN_5O$	8.8[f]	8.9	25	58[e]
3	H	Th[g]	129	$C_{13}H_{17}HgN_9O_2$	23.0	23.0	50	78 (6.2)
4	CH_3	OAc	181	$C_9H_{15}HgN_5O_2$	15.8	15.9	50	62 (8.6)
5	CH_3	Th[g]	117	$C_{14}H_{19}HgN_9O_2$	22.3	22.2	40	119 (4.9)
6	CH_3	CS[h]	113	$C_{13}H_{16}HgN_6O_4$	15.5	15.6	50	99 (7.4)
7	CH_3	MCS[i]	108	$C_{16}H_{18}HgN_6O_4$	..	..	30	104 (5.8)
8	CH_3	KF_2[j]	148	$C_7H_{16}F_2HgKN_5O_3$	14.1	13.4	50	81 (5.1)
9	CH_3	Asc[k]	108	$C_{13}H_{19}HgN_5O_7$	12.5	12.7	..	.....
10	C_2H_5	OAc	122	$C_{10}H_{17}HgN_5O_2$	15.4	15.4	..	92 (6.2)
11	C_2H_5	Th[g]	110	$C_{15}H_{21}HgN_9O_2$	21.9	21.6	100	64 (8.6)
12	n-C_3H_7	Br	168	$C_9H_{16}BrHgN_5O$	14.3	14.1	..	.....
13	n-C_3H_7	Th[g]	182	$C_{16}H_{23}HgN_9O_2$[l]	..	..	100	50
R_1 = CH_3, R_2 = H								
14	CH_3	OAc	125	$C_{10}H_{17}HgN_5O_2$	15.3	14.7	..	.....
15	CH_3	Th[g]	108	$C_{15}H_{23}HgN_9O_3$[m]	20.5	21.3	50	106 (3.3)
16	CH_3	KF_2[j]	115	$C_8H_{15}F_2HgKN_5O_3$	13.7	13.7	..	.....
R_1 = H, R_2 = CH_3								
17	H	OAc	132	$C_9H_{13}HgN_5O_2$[n]	..	..	30	58[e]
18	H	Cl	212	$C_7H_{12}ClHgN_5O$	16.7	16.1	20	44[e]
19	H	Br	209	$C_7H_{12}BrHgN_5O$	17.3[o]	18.1	30	42[e]
20	CH_3	OAc	149	$C_{10}H_{17}HgN_5O_2$	15.4	14.8	25	72[e] (6.4)
21	CH_3	Cl	179	$C_8H_{14}ClHgN_5O$	8.2[f]	8.5	20	50[e]
22	CH_3	Th[g]	124	$C_{15}H_{21}HgN_9O_2$	21.9	21.8	20	51[e]
3-Chloromercuri-2-methoxypropyl urea							60	97/61[e] (10.2/7.4)
Mercurophylline								48 (3.2)
Control test (no drug given)								43 (4.9)

[a] Melting points are all with decomposition and are not corrected. All of the melting points of these mercurials, and those listed in the experimental section were characterized by long initial range of faint darkening and a point of definitive darkening followed by a long range of gross decomposition. The point of definitive darkening has been listed as the melting point. The compounds would not recrystallize from the usual solvents, although most of them were very soluble in water. [b] Analyses by Weiler and Strauss, Oxford, England. [c] The acute LD min. (minimal lethal dose) was established in mice by subcutaneous administration of a solution of the compound and is expressed in mg./Kg. (8–16 mice per test). [d] Screening for diuretic activity was established in dogs by intravenous injection using the calculated amount of compound to give a test level of 0.5 mg. mercury/Kg. The dogs were dehydrated for twenty-four hours and catheterized just before testing; they were then force fed 0.9% saline at the level of 25 ml./Kg. and the compound administered. The volume of urine voided was collected at five hours post-injection and the activity (A) expressed as percentage diuresis calculated on the basis of the following formula: Diuresis, % = (Urine volume at 5 hr./Saline volume given at 0 hr.) × 100. Each result is expressed as the average response of six dogs. [e] Compound evaluated at 0.25 mg. mercury/Kg. Standard error = (S. E.). [f] Chlorine analysis. [g] 7-Theophylline derivative. [h] 3-(Carbonylsalicylamide) derivative. [i] 3-(8-Methylcarbonylsalicylamide) derivative.—Calcd.: C, 34.4; H, 3.2. Found: C, 35.4; H, 3.6. [j] Dihydrate, compd. 8—Calcd.: C, 17.0; H, 3.2. Found: C, 17.0; H, 3.5. Compd. 16—Calcd.: C, 18.8; H, 3.5. Found: C, 18.7; H, 3.5. [k] 1-Ascorbyl derivative, nature of bonding not established. [l] Calcd.: C, 32.6; H, 3.9. Found: C, 31.8; H, 4.0. [m] Dihydrate—Calcd.: C, 29.3; H, 4.1. Found: C, 29.0; H, 4.2. [n] Calcd.: C, 24.5; H, 3.4. Found: C, 24.3; H, 3.8. [o] Bromine analysis—At 5 mg. mercury/Kg. orally in two dogs, % diuresis (five hours), compd. 5, 113%; 3-chloromercuri-2-methoxypropyl urea, 122%.

EXPERIMENTAL[1]

Allylbiguanide Hydrochloride.—A mixture of 94.7 Gm. (1 mole) of allylamine hydrochloride and 84 Gm. (1 mole) of dicyandiamide was fused over a four-hour period in an oil bath maintained at 135–165°. The solid reaction product obtained after cooling was recrystallized from 500 ml. of boiling ethanol (carbon) yielding 83.1 Gm. (47%), m. p. 171–181°. Recrystallization from propanol gave m. p. 178–179°.

Anal.—Calcd. for $C_5H_{12}ClN_5$: C, 33.8; H, 6.8. Found: C, 33.4; H, 7.2.

[1] Descriptive data shown in the table is not reproduced in the Experimental Section.

N-Allylformoguanamine (from Allylbiguanide Hydrochloride).—A solution of 6.0 Gm. (0.033 mole) of allylbiguanide hydrochloride in 30 ml. of methanol was treated with a solution of 0.8 Gm. (0.033 mole) of sodium in 20 ml. of methanol. Ethyl formate (3.5 ml.) was added to the cooled solution and the reaction mixture maintained at 20° for seventy-two hours. After addition of 100 ml. of water and removal of the methanol, 4.4 Gm. of product separated. Recrystallization from acetonitrile gave 2.3 Gm. (46%), m. p. 148–149° (28).

Anal.—Calcd. for $C_6H_9N_5$: C, 47.7; H, 6.0. Found: C, 47.5; H, 5.9.

The picrate prepared from a methanol solution

with aqueous picric acid, recrystallized from water, melted at 194–195°.

Anal.—Calcd. for $C_{12}H_{12}N_8O_7$: C, 37.9; H, 3.2. Found: C, 37.6; H, 3.2.

N-Allylformoguanamine (from Allylamine Hydrochloride).—Fusion of allylamine hydrochloride and dicyandiamide as above was effected. The reaction mass was solubilized in methanol . equivalent quantity of sodium methoxide adde ., followed by ethyl formate and the N-allylformoguanamine isolated as above in 50% yield, m. p. 146–147° (isopropanol), not depressing the melting point of the compound isolated starting with pure allylbiguanide hydrochloride; mixed m. p. 146–147°.

N-Allylacetoguanamine.—To a suspension of 35.5 Gm. (0.2 mole) of allylbiguanide hydrochloride in 100 ml. of acetonitrile was added a solution of 16.8 Gm. (0.4 mole) of sodium hydroxide in 100 ml. of water. The two-phase system was stirred and cooled (5°) during the addition of 17 Gm. (0.22 mole) of acetyl chloride over thirty minutes. Stirring at 20° was continued for three hours. After removal of acetonitrile and addition of 100 ml. of water, 26.6 Gm. of product separated which after drying and recrystallization from benzene gave 19 Gm. (58%), m. p. 113–116°.

Anal.—Calcd. for $C_7H_{11}N_5$: C, 50.9; H, 6.7; N, 42.4. Found: C, 51.1; H, 6.8; N, 42.3.

The picrate prepared from a methanol solution with aqueous picric acid melted at 187–189° (methanol).

N-Methallylaminoformoguanamine.—A mixture of 18.7 Gm. (0.17 mole) of methallylamine hydrochloride and 12.3 Gm. (0.17 mole) of dicyandiamide was fused over a two-hour period in an oil bath maintained at 150–180°. The cooled fusion product was dissolved in a solution of 4.6 Gm. (0.2 mole) of sodium in 140 ml. of methanol. Ethyl formate (15 ml.) was added to the cooled solution and the reaction mixture maintained at 20° for seventy-two hours. The sodium chloride was removed and the filtrate evaporated. The solid residue was extracted with three 250-ml. portions of hot benzene. The combined benzene extracts on standing gave 9.9 Gm. (35%), m. p. 132–134°.

Anal.—Calcd. for $C_7H_{11}N_5$: C, 50.9; H, 6.7. Found: C, 51.3; H, 6.8.

2-Amino-N-4-(3′-acetoxymercuri-2′-methoxypropyl)amino-*s*-triazine (Compound 4).—To a solution of 9.0 Gm. (0.06 mole) of N-allylformoguanamine dissolved in 90 ml. of methanol was added a hot solution of 16.8 Gm. (0.053 mole) of mercuric acetate in 3 ml. of glacial acetic acid and 42 ml. of methanol. The reaction mixture was stirred under reflux for twenty hours and cooled. The volatiles were removed *in vacuo*, the product separated, triturated with acetone, and dried over phosphorus pentoxide. There was obtained 15.0 Gm. (57%).

2-Amino-N-4-(3′-[7-theophylline]mercuri-2′-methoxypropyl)amino-*s*-triazine (Compound 5).—Three grams (0.0068 mole) of compound 4 was added portionwise to a suspension of 1.28 Gm. (0.007 mole) of anhydrous theophylline in 25 ml. of water. As the compound is formed, complete solution is effected. The reaction mixture was filtered to remove traces of insoluble material. Removal of filtrate water *in vacuo* yielded a residue of the product which was slurried with acetone, separated and dried over phosphorus pentoxide and weighed 3.25 Gm. (84%). One gram of compound 5 dissolves in less than 1 ml. of water.

2-Amino-N-4-(3′-[3-(8-methylcarbonylsalicylamido)]mercuri-2′-methoxypropyl)amino-*s*-triazine (Compound 7).—Three grams (0.0068 mole) of compound 4 was added portionwise to a suspension of 1.27 Gm. (0.0072 mole) of 8-methylcarbonylsalicylamide in 75 ml. of water and 30 ml. of ethanol with warming to 60°. The reaction mixture was filtered to remove traces of insoluble material and the filtrate concentrated to a thick syrup *in vacuo*. After trituration with acetone and drying over phosphorus pentoxide, 2.9 Gm. (74%) of product was obtained.

2-Amino-N-4-(3′-hydroxymercuri-2′-methoxypropyl)amino-*s*-triazine (Compound 23).—The reaction mixture was set up as for the preparation described under compound 4. The methanol solution of the formed compound 4 was treated with cooling and stirring with a solution of 6.5 Gm. of sodium hydroxide in 40 ml. of methanol. The copious white precipitate was filtered and dried, 22.9 Gm. (95%), m. p. 170° (dec.). The product could not be recrystallized nor obtained analytically pure but was used directly for preparation of the compounds 9 and 10.

Anal.—Calcd. for $C_7H_{13}HgN_5O_2$: C, 21.0; H, 3.2. Found: C, 22.2; H, 3.0.

2-Amino-N-4-(3′-ascorbylmercuri-2′-methoxypropyl)amino-*s*-triazine (Compound 9).—To a gummy suspension of 6.4 Gm. (0.016 mole) of compound 23 in 20 ml. of water there was added portionwise 3.17 Gm. (0.018 mole) of *l*-ascorbic acid. After one hour, the solution obtained was treated with carbon, filtered, and the water removed *in vacuo*. The light yellow solid was dried over phosphorus pentoxide, triturated with acetone, and redried. There was obtained 7.7 Gm. (77%) of product as a white powder.

2-Amino-N-4-(3′-fluoromercuri-2′-methoxypropyl)amino-*s*-triazine potassium fluoride dihydrate (Compound 8).—To a gummy suspension of 6.4 Gm. (0.016 mole) of Compound 23 in 20 ml. of water there was added portionwise 1.4 Gm. (0.018 mole) of potassium acid fluoride. After trituration, stirring and standing for one hour, the components of the reaction mixture were substantially dissolved. Carbon was added and the solution filtered. Evaporation of the water *in vacuo* yielded a yellow syrup which solidified after drying for fifteen hours over phosphorus pentoxide. Upon trituration with acetone, there was obtained 6.1 Gm. (68%) of product as a light yellow powder.

Tris(3′-hydroxymercuri-2′-methoxypropyl)cyanurate (Compound 24).—A stirred solution of 20.0 Gm. (0.08 mole) of tri-allylcyanurate in 250 ml. of methanol was treated portionwise with 76.5 Gm. (0.24 mole) of mercuric acetate and allowed to stand twenty hours. The clear solution was stirred and treated with a solution of 9.6 Gm. (0.24 mole) of sodium hydroxide in 100 ml. of methanol. The formed precipitate (34 Gm.) was separated, and concentration of the filtrate to 75 ml. afforded an additional 43.5 Gm. of product (total yield, 97.5%). The product melted at 225° (dec.).

Anal.—Calcd. for $C_{15}H_{27}Hg_3N_3O_9$: C, 18.1; H, 2.7; N, 4.2. Found: C, 18.3; H, 2.86; N, 4.6.

Tris(3′-hydroxymercuri-2′-ethoxypropyl)cyanurate (Compound 25).—Substitution of ethanol as the solvent medium in the reaction described above gave 40.3 Gm. (51%) of product, m. p. 250–280° (dec.).

Anal.—Calcd. for $C_{18}H_{33}Hg_3N_3O_9$: N, 4.1. Found: N, 4.0.

Tris(3′-[7-theophylline]mercuri-2′-methoxypropyl)cyanurate (Compound 26).—To a warmed solution of 3.2 Gm. (0.018 mole) of theophylline in 200 ml. of water was added 6.0 Gm. (0.006 mole) of compound 24. The clear solution was allowed to stand overnight. The water was removed *in vacuo* and the residue of product dried over phosphorus pentoxide weighed 8.0 Gm. (90%), m. p. 194–222° (dec.).

Anal.—Calcd. for $C_{36}H_{45}Hg_3N_{15}O_{12}$: C, 29.2; H, 3.0; N, 14.2. Found: C, 29.1; H, 3.4; N, 14.4.

Tris(3′-[7-theophylline]mercuri-2′-ethoxypropyl)cyanurate (Compound 27).—Processed as indicated for compound 26 using compound 25 as the initial reactant, the product was obtained in 84% yield, m. p. 250° (dec.).

Anal.—Calcd. for $C_{39}H_{51}Hg_3N_{15}O_{12}$: N, 13.8. Found: N, 13.8.

Compound 26 was evaluated (intraperitoneal) in rats following the method of Lipschitz (29). Twelve rats were used in each group and dosage levels employed were compound 26, 12 mg./Kg. (Groups I and II); mercurophylline, 12 mg./Kg. (Group III); and urea, 1,500 mg./Kg. (Group IV). The results are summarized in Table II.

TABLE II.—DIURETIC FACTOR[a] IN HOURS

Group	Hours 1	2	3
I[b]	5.10	2.14	1.43
II[b]	7.40	1.85	1.60
III[b]	1.93	1.33	1.12
IV	1.47	1.44	1.44

[a] Diuretic factor = Urine collected in test group/Urine collected in control group.
[b] Level of mercury in mg./Kg. corresponding to dosage used: I and II, 4.9; III, 4.7.

SUMMARY

1. The diuretic response in dogs, upon intravenous injection of a variety of newly synthesized mercurated amino-triazines, has been assessed in terms of relation of structure to activity.

2. Structural features in I associated with the maximal diuretic response are Y = methyl, R_1 = hydrogen, R_2 = hydrogen and X = theophylline, chloride, carbonylsalicylamide, or 8-methylcarbonylsalicylamide.

3. Synthesis of the required intermediates for ultimate mercuration, such as 2-allylamino-4-amino-*s*-triazine, 2-amino-4-methallylamino-*s*-triazine, and 2-allylamino-4-amino-6-methyl-*s*-triazine is described.

4. An additional series of mercurial diuretics prepared by mercuration of tri-allylcyanurate is described.

REFERENCES

(1) Stroud, W. D., and Wagner, J. A., *Med. Clin. N. Amer.*, **38,** 431(1954).
(2) (a) Vogl, A., *Am. Practitioner and Dig. Treatment*, **5,** 356(1954); (b) Smallwood, W. C., and Matthews, H. L., *Lancet*, **1,** 121(1956); (c) Segal, R. L., *Ann. Internal Med.*, **43,** 435(1955).
(3) Kaufman, J. G., Bernstein, A., Weiss, F., Klosk, E., and Feldman, H. S., *Circulation*, **12,** 52(1955).
(4) Vogl, A., *Am. Heart J.*, **39,** 881(1950).
(5) (a) Schlesinger, A., Weiner, N., and Gordon, S. M., U. S. Patent 2,728,780; (b) Wendt, G. R., U. S. Patent 2,751,325.
(6) (a) Halpern, A., U. S. Patent 2,592,418; (b) Foye, W. O., and Mode, R. A., THIS JOURNAL, **44,** 76(1955); (c) Werner, L. H., and Scholz, C. R., *J. Am. Chem. Soc.*, **76,** 2453(1954); (d) Lehman, R. A., U. S. Patents 2,576,349 and 2,675,388.
(7) (a) Barry, R. H., Wang, S. M., and Walter, L. A., U. S. Patent 2,749,339; (b) Ehrhart, G., *et al.*, U. S. Patent 2,704,767.
(8) (a) Rowland, R. L., Perry, W. L., Foreman, E. L., and Friedman, H. L., *J. Am. Chem. Soc.*, **72,** 3595(1950); (b) Rowland, R. L., Perry, W. L., and Gerstein, S., *ibid.*, **73,** 3691(1951).
(9) Shelton, R. S., Farmer, J. L., and Tilford, C. H., U. S. Patent 2,601,461.
(10) (a) Batterman, R. C., *Am. Heart J.*, **48,** 780(1954); (b) Dimitroff, S. P., Lewis, R. C., Thorner, M. C., and Field, J. B., *ibid.*, **49,** 407(1955); (c) Moyer, J. H., McConn, R. G., Seibert, R. A., Dennis, E. W., and Hughes, W., *J. Chronic Diseases*, **2,** 670(1955).
(11) Lipschitz, W. L., and Stokey, E., *J. Pharmacol.*, **83,** 235(1945).
(12) Lipschitz, W. L., and Hadidian, Z., *ibid.*, **81,** 84(1944).
(13) (a) Sugino, K., and Idzumi, S., *J. Chem. Soc. Japan*, **65,** 265(1944); *Chem. Abstr.*, **41,** 3762(1947); (b) Bobeck, F., *Ann.*, **487,** 294(1931); (c) Tendick, F. H., and Burckhalter, J. H., *J. Am. Chem. Soc.*, **72,** 1862(1950); (d) Detweiler, W. K., and Amstutz, E. D., *ibid.*, **74,** 1482(1952).
(14) Overberger, C. G., and Shapiro, S. L., *ibid.*, **76,** 93(1954).
(15) Overberger, C. G., and Shapiro, S. L., *ibid.*, **76,** 1061(1954).
(16) (a) Pearson, D. E., Sigal, M. V., Jr., and Krug, R. H., *J. Org. Chem.*, **15,** 1048(1950); (b) Pearson, D. E., and Sigal, M. V., Jr., *ibid.*, 1055(1950); (c) Park, W. R. R., and Wright, G. F., *ibid.*, **19,** 1435(1954); (d) Chatt, J., *Chem. Rev.*, **48,** 7(1951).
(17) Shapiro, S. L., Rose, I. M., and Freedman, L., Atlantic City Meeting, American Chemical Society, September 1956.
(18) Degraff, A. C., and Lehman, R. A., *J. Am. Med. Assoc.*, **119,** 998(1942).
(19) (a) Shaffer, C. F., *ibid.*, **124,** 700(1944); (b) Tacket, H. S., and Gubin, M. L., *J. Clin. Invest.*, **33,** 844(1954).
(20) Benesch, R., and Benesch, R. E., *Circulation*, **14,** 910(1956).
(21) Mustakallio, K. K., and Telkkä, A., *Ann. Med. Exp. et Biol. Fenniae(Helsinki)*, **33,** 3(1955).
(22) Blumberg, H., Schlesinger, A., and Gordon, S. M., *J. Pharmacol. Exptl. Therap.*, **105,** 336(1952).
(23) Friedman, H. L., Cincinnati Meeting, American Chemical Society, April 1955.
(24) Halpern, A. L., Jones, J. W., and Gross, E. G., THIS JOURNAL, **37,** 333(1948).
(25) McColl, J. D., Parker, J. M., and Ferguson, J. K. W., *Can. J. Biochem. Physiol.*, **34,** 903(1956).
(26) Yale, H. L., U. S. Patent 2,672,472.
(27) Nadkarni, M. K., and Jones, J. W., THIS JOURNAL, **39,** 297(1950).
(28) Swiss Patent 261,812; *Chem. Abstr.*, **44,** 4517(1950); reported m. p. 152.5°.
(29) Lipschitz, W. L., Hadidian, Z., and Kerpscar, A., *J. Pharmacol. Exptl. Therap.*, **79,** 97(1943).

Investigation and Development of Protective Ointments I.*

Development of a Method for Measuring Permeation of Mechanical Barriers

By L. M. LUECK,† D. E. WURSTER, T. HIGUCHI, A. P. LEMBERGER, and L. W. BUSSE

A semiquantitative method has been developed for measuring permeation of semisolid materials by true diffusion. Adherence to theory has been demonstrated using aniline, nitrobenzene, and isovaleric acid as penetrants and white petrolatum and Jelene[1] as barriers. Application of this technique to the study of penetration of protective films by noxious chemicals gives an index to protective capacity.

INTACT SKIN serves as a highly efficient device in protecting underlying body tissues. However, certain chemical and physical agents have the ability to penetrate through this protective covering (1–4) and produce either local or systemic manifestations of their toxicity (5–8). For this reason man has made continual efforts to fabricate pharmaceutical preparations designed to augment and reinforce the protective properties of the skin. In the usual case these preparations take the form of ointments. The purpose of this study is to investigate these pharmaceutical forms in an effort to obtain information on how they function, thus making possible the formulation of better protective preparations.

Although formulation of protective creams has often been based principally upon empirical methods, some attempts have been made to test the ability of the ointment to fulfill its purpose. This testing has involved both *in vivo* and *in vitro* types of experimentation.

Sulzberger has done considerable work employing *in vivo* testing procedures, particularly in evaluating the relative protective and decontaminating properties of various BAL ointments (9). *In vivo* testing procedures have also been used with some success in evaluating flash burn preventive creams (10).

More work has been done in evaluating protective ointments where *in vitro* methods of testing could be adapted. Schwartz, Mason, and Albritton (11) developed a technique to measure the permeability of protective ointments in which an ointment film was prepared between sheets of filter paper and the time required for various penetrants to pass through the film was determined by pH indicating chemicals. Eisner (12) employed the same technique but modified it by using sea sand and kaolin as support material in the barrier to accommodate liquids. Neither of these studies attempted to investigate the diffusional pattern followed in the permeation process.

In the present study a technique has been developed for measuring the diffusion of chemical agents through a protective barrier.

THEORETICAL CONSIDERATIONS

The penetration of an ointment film such as is applied to the skin when using protective ointments occurs in two phases. Initially, the penetrating agent partitions between its carrying medium, be it solid, liquid, or gas, and the exposed surface layer of the ointment barrier. This is followed by the second phase of penetration, the diffusion of the penetrating agent through the ointment film.

The second phase of permeation may be further subdivided into two stages. The first stage is the establishment of a uniform concentration gradient of penetrant across the barrier, and the second is the constant, uniform diffusion of penetrant through the barrier after the uniform concentration gradient has been established. This second stage is known as steady-state diffusion.

According to Fick's first law of diffusion, which describes steady-state diffusion,

$$Q = -D(dc/dx) \qquad \text{(Eq. 1)}$$

where Q is the flux or amount of material crossing a plane of unit area per unit time, dc/dx is the concentration gradient and D is the diffusion coefficient.

Since the steady state is difficult to achieve experimentally, the concept of quasi-steady state has arisen. This concept allows treatment of those cases which nearly meet steady state requirements, that is, situations where the concentration gradient

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[1] Research Products Corp. (Plastibase—E. R. Squibb and Sons).

changes only slightly with time. Where this change is small enough, the assumption of a constant concentration gradient does not give rise to serious error.

Quasi-steady state diffusion can be applied to the situation wherein two well-stirred liquids, either or both containing a solute, are separated by an ointment barrier permeable only to the solute. If A is the cross-sectional area and L the depth of the barrier, the flux is given by

$$Q = AD(C_a' - C_b')/L \qquad \text{(Eq. 2)}$$

where C_a' and C_b' are the concentrations of the solute in the respective surfaces of the barrier, C_a' being the concentration on the side from which solute flows, after a quasi-steady state is established. If C_a is the concentration of solute in solution on side a, C_b the concentration on side b, and V_a and V_b are the volumes of the solutions on the respective sides of the barrier, it follows that

$$Q = V_b dC_b/dt = -V_a dC_a/dt = AD(C_a' - C_b')/L \qquad \text{(Eq. 3)}$$

The distribution coefficient, (DC), of the solute between the solvent and barrier is given by

$$DC = C_a'/C_a = C_b'/C_b$$

so that on substituting into Eq. 3 the equation becomes

$$dC_b/dt = AD(DC)(C_a - C_b)/V_bL \qquad \text{(Eq. 4)}$$

If T is the total amount of solute in the system and the amount in the barrier initially is assumed to be negligible

$$T = C_aV_a + C_bV_b$$

so that

$$C_a = (T - C_bV_b)/V_a$$

On substitution into Eq. 4 the expression becomes

$$dC_b/dt = AD(DC)/V_bL \times [T/V_a - C_b(V_b + V_a)/V_a] \qquad \text{(Eq. 5)}$$

In the special case where $V_a = V_b$ and initially $C_b = 0$ this equation reduces to

$$dC_b/dt = AD(DC)[T/V - 2C_b]/VL \qquad \text{(Eq. 6)}$$

In this case T/V = initial concentration of solute on side $A = C_0$. If the term $AD(DC)/VL$ is expressed as a constant, K, on integration the equation becomes

$$\log(C_0 - 2C_b) = (-2K/2.303)t + \log C_0 \qquad \text{(Eq. 7)}$$

provided D can be assumed constant over the concentration range employed.

Equation 7 is a convenient form for describing the diffusional process under quasi-steady state conditions. By determining C_0, C_b, and t experimentally and plotting $\log(C_0 - 2C_b)$ as a function of t, a straight line is obtained, the slope of which is equal to $-2K/2.303$. In the case of penetration of protective films, K may be regarded as a permeability constant and serves to indicate the rate at which a penetrant material passes through the barrier after a uniform concentration gradient has been established.

EXPERIMENTAL

Apparatus.—The apparatus employed to test the resistance of an ointment to a given agent is shown in Fig. 1. A similar assembly using a polyethylene mounting was also employed but it was found that

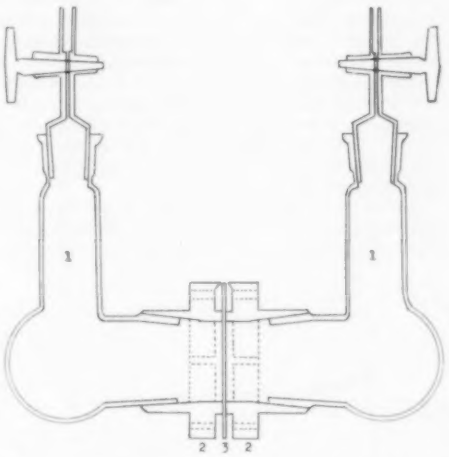

Fig. 1.—Barrier cell apparatus. 1—Glass chamber, 2—Aluminum mounting, 3—Bronze washer.

certain agents such as nitrobenzene were capable of partitioning into the polyethylene, thus giving rise to error in the determination.

Procedure.—To prepare the ointment barrier, a thin layer of stopcock grease was spread on the bronze washer, a silk disk was placed in position over the hole on one side of the bronze washer and the cavity in the bronze washer was filled with ointment. A second silk disk was then placed over the ointment and the excess ointment forced out by slowly rolling a 30-pound steel cylinder over this part of the assembly. The barrier thus prepared was placed in the barrier cell mounting and secured with four bolts. The glass chambers were then fitted to the mounting. All joints were sealed with a thin layer of stopcock grease.

Exactly 40 or 50 ml. of a solution containing the penetrant substance was introduced into one chamber with the simultaneous addition of an equal volume of solvent into the other chamber. The assembly was then placed in a constant temperature bath at 25°, the ground glass stoppers positioned, and the entire apparatus subjected to a mild, continuous shaking. At appropriate time intervals, 0.5- or 1.0-ml. samples were withdrawn from each chamber and analyzed for content of penetrating agent.

The penetrant substances and solvent systems employed were aniline in water containing 1% sodium sulfite as antioxidant, nitrobenzene in 51.4% w/w ethanol, and isovaleric acid in 0.001 M aqueous sulfuric acid. Barrier materials were white petrolatum and Jelene.

RESULTS AND DISCUSSION

The technique employed in this study was devised to measure the penetration of ointment barriers by true diffusion, thus it was necessary to establish that the procedure followed for preparing the barriers led to the formation of a continuous film of ointment. For this reason a number of barriers were prepared and subjected to a pressure differential of 8 mm. Hg. It was found that the barriers prepared as described were capable of maintaining this pressure differential for a minimum of eight hours. A second test for continuity consisted of exposing a number of barriers to 0.5 *N* hydrochloric acid solution in the barrier cell apparatus. After twenty-two hours it was impossible to detect hydrochloric acid in the opposite chamber of the apparatus thus indicating that the barriers prepared as described above were continuous.

Sample plots for the penetration of barriers composed of Jelene and white petrolatum by aniline and nitrobenzene are shown in Fig. 2. In these plots

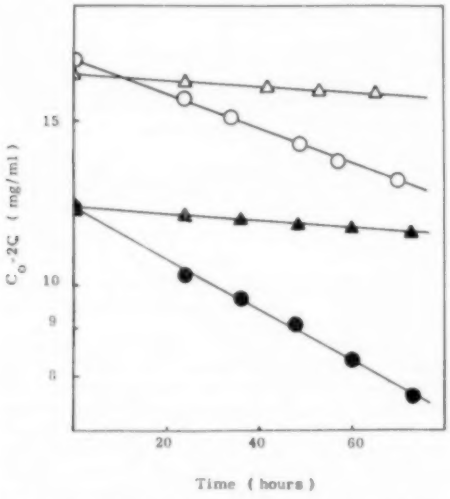

Fig. 2.—Sample plots for penetration of Jelene and white petrolatum barriers by aniline and nitrobenzene: △-white petrolaatum-aniline, ○—Jelene-aniline, ●—Jelene-nitrobenzene, ▲—white petrolatum-nitrobenzene.

$\log (C_o - 2C_b)$ has been plotted as a function of the time of sampling. From the slopes of these and similar plots, K values have been calculated for the penetration of these barriers by these agents and are listed in Tables I and II.

Both of these penetrating agents serve as examples for the penetration of an ointment film by monomolecular diffusion. As is expected from the equation describing this situation, the permeation constant is shown to be essentially independent of the initial concentration of penetrant material in the solvent employed. It is also of interest to note that with both penetrating agents the permeability constant was slightly greater for the Jelene barrier

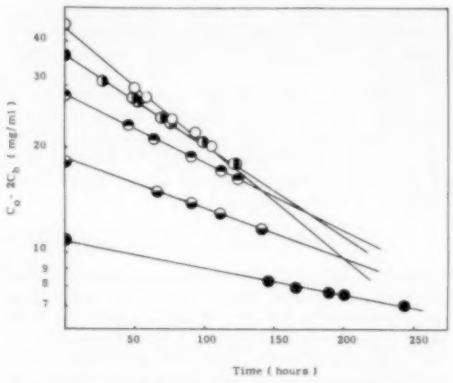

Fig. 3.—Sample plots for penetration of Jelene barrier by isovaleric acid. ○—C_0 = 44.7 mg./ml., ◑—C_0 = 36.7 mg./ml., ◒—C_0 = 28.1 mg./ml., ◒—C_0 = 18.2 mg./ml., ●—C_0 = 10.9 mg./ml.

TABLE I.—DATA FOR PENETRATION OF JELENE AND WHITE PETROLATUM BARRIERS BY ANILINE

Jelene		White Petrolatum	
C_o (mg./ml.)	$K \times 10^3$	C_o (mg./ml.)	$K \times 10^3$
17.36	2.5	17.27	0.28
17.36	2.4	17.27	0.27
3.89	2.7	8.23	0.29
3.89	2.5	8.23	0.29
3.89	2.7	8.23	0.30
13.03	2.7	12.20	0.31
13.03	2.7	12.20	0.30
13.03	2.9	16.80	0.29
14.92	2.8	16.80	0.32
14.92	2.8	27.00	0.28
...	...	27.00	0.27

TABLE II.—DATA FOR PENETRATION OF JELENE AND WHITE PETROLATUM BARRIERS BY NITROBENZENE

Jelene		White Petrolatum	
C_o (mg./ml.)	$K \times 10^3$	C_o (mg./ml.)	$K \times 10^3$
23.67	2.6	2.94	0.44
23.67	2.7	2.94	0.46
23.67	3.0	2.94	0.46
12.11	2.9	12.11	0.43
12.11	2.9	12.11	0.45
12.11	3.3	12.11	0.55
4.73	2.7	3.80	0.45
4.73	2.6	3.80	0.57
4.73	2.7	3.80	0.58
1.25	2.7	3.80	0.46
1.25	2.6	1.25	0.46
1.25	3.2	1.25	0.45
2.02	2.8	1.25	0.47
2.02	2.7	2.02	0.47
2.02	2.8	2.02	0.55
...	...	2.02	0.68

than for the white petrolatum barrier. Since Jelene consists of mineral oil gelled with polyethylene, molecular diffusion through this medium may be considered essentially the same as through

TABLE III.—DATA FOR PENETRATION OF JELENE AND WHITE PETROLATUM BARRIERS BY ISOVALERIC ACID

Jelene			White Petrolatum		
C_o (mg./ml.)	$K \times 10^4$	$Z \times 10^6$	C_o (mg./ml.)	$K \times 10^4$	$Z \times 10^6$
10.89	9	8.3	11.02	1.5	1.3
10.89	9	8.3	11.02	1.4	1.3
10.89	9	8.3	11.02	1.5	1.3
18.19	15	8.3	11.02	1.5	1.4
18.19	16	8.6	11.02	1.5	1.4
18.19	17	9.4	18.03	2.4	1.4
28.06	23	8.2	18.03	2.5	1.4
28.06	24	9.4	18.03	2.5	1.4
28.06	24	8.6	19.12	2.4	1.4
36.74	30	8.3	19.12	2.4	1.3
36.74	31	8.4	19.12	2.4	1.3
44.68	37	8.3	19.12	2.5	1.3
44.68	37	8.2	19.12	2.5	1.3
44.68	39	8.7	22.86	3.2	1.4
			22.86	2.9	1.3
			22.86	3.0	1.3
			30.25	4.0	1.3
			30.25	4.4	1.4
			30.25	4.3	1.4
			33.27	5.2	1.6
			33.27	5.0	1.5
			33.27	5.1	1.5
			33.27	5.1	1.5
			33.27	5.1	1.5

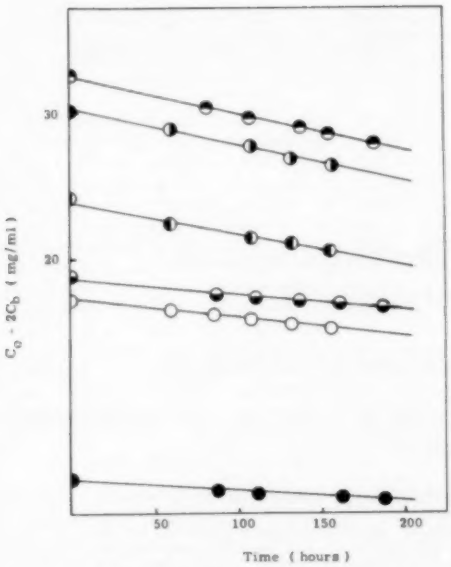

Fig. 4.—Sample plots for penetration of white petrolatum barrier by isovaleric acid. ◓—C_o = 33.3 mg./ml., ◑—C_o = 30.3 mg./ml., ◐—C_o = 22.9 mg./ml., ◒—C_o = 18.0 mg./ml., ●—C_o = 11.0 mg./ml., ○—C_o = 19.1 mg./ml.

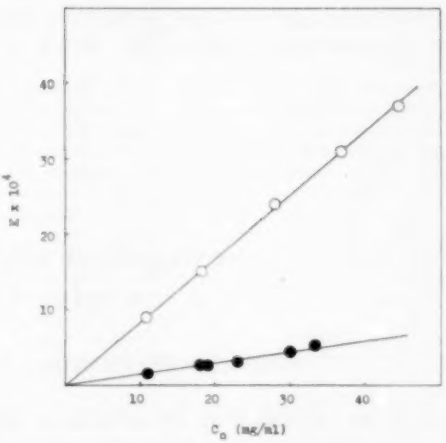

Fig. 5.—Relationship between initial concentration and permeability constant for dimeric diffusion. ●—white petrolatum barrier, ○—Jelene barrier.

mineral oil. According to the Stokes-Einstein equation, the diffusion coefficient varies inversely with the viscosity, thus it follows that the penetration of the Jelene barrier should occur more readily.

Similar sample plots to the above have been made for the penetration of Jelene and white petrolatum barriers by isovaleric acid. These plots are shown in Figs. 3 and 4, and the K values for all experimental data are given in Table III.

The solvent for isovaleric acid was 0.001 M sulfuric acid. In this solvent the isovaleric acid existed in the monomeric form, but in the nonpolar ointment barrier it was expected to dimerize thus serving as an example of diffusion by a dimer.

In such a case the distribution coefficient = (C_a'/C_a^2) so that the equation

$$dC_b/dt = K(C_a^2 - C_b^2) \qquad \text{(Eq. 8)}$$

is used to describe bimolecular diffusion. Following a derivation scheme similar to that for unimolecular diffusion, the equation

$$\log (C_o - 2C_b) = -2ZC_o/2.303\,t + \log C_o \quad \text{(Eq. 9)}$$

can be derived where Z is a coefficient of permeability for this particular case. Comparison of this equation with Eq. 7 shows that

$$Z = K/C_o \quad \text{(Eq. 10)}$$

Calculated values for Z are listed in Table III. In Fig. 5 the expected linear relationship between C_o and K is seen to hold for the penetration of both Jelene and white petrolatum.

These results strongly support the conclusion that the method outlined is satisfactory for studying the diffusion of penetrating agents through semisolids. Further, the results obtained with nitrobenzene are not conclusive as to whether diffusion occurs through pinholes or across the continuous medium under the described experimental conditions. However, the linear relationship between C_o and K for isovaleric acid penetration lends evidence for the conclusion that the latter is the case in these studies.

SUMMARY

A method for measuring permeation by true diffusion has been developed which gives rise to semiquantitative results. The method is relatively simple and can be modified easily to accommodate the study of a wide variety of materials.

By applying this technique to the evaluation of protective barriers, the protective capacity of a given ointment material can be represented in terms of a permeability constant. Comparison of the permeability constants obtained for various barrier materials makes it possible to classify these materials according to their relative protective capacities.

The data obtained by this technique have been evaluated and shown to follow the theoretical predictions.

REFERENCES

(1) Hermann, F., *N. Y. State J. Med.*, **44**, 2452(1944).
(2) MacKee, G. M., Sulzberger, M. B., Hermann, F., and Baer, R. L., *J. Invest. Dermatol.*, **6**, 43(1945).
(3) Rothman, S. J., *J. Lab. Clin. Med.*, **8**, 1305(1943).
(4) Rothman, S. J., and Flesch, P., *Ann. Rev. Physiol.*, **6**, 195(1944).
(5) Walzer, A., *Arch. Dermatol. and Syphilol.*, **41**, 692 (1940).
(6) *Ibid.*, **49**, 427(1944).
(7) Miller, O., *J. Invest. Dermatol.*, **12**, 19(1949).
(8) Luduena, F. P., *Arch. Dermatol. and Syphilol.*, **57**, 210(1948).
(9) Sulzberger, M. B., *ibid.*, **56**, 90(1947).
(10) Glickman, C. S. *Drug & Cosmetic Ind.*, **57**, 328(1945).
(11) Schwartz, L., Mason, H. S., and Albritton, H., *Occupational Med.*, **1**, 376(1946).
(12) Eisner, H., *J. Invest. Dermatol.*, **10**, 273(1948).

Investigation and Development of Protective Ointments II.*

Influence of Partition Coefficient and Thickness

By L. M. LUECK, D. E. WURSTER, T. HIGUCHI, K. F. FINGER, A. P. LEMBERGER, and L. W. BUSSE

A method suitable for measuring the permeation of continuous ointment films by diffusional processes when the penetrating agent is in solution in a liquid contacting the film has been modified to allow the study of penetration by substances in the vapor state. It has been shown that in both liquid-contact and gaseous penetration, the permeation constant is related directly with the distribution coefficient and inversely with the thickness of the film. These findings confirm the validity of the method as a means of studying the penetration of ointment films by true diffusion and indicate that the selection of a semisolid protective material for a given penetrating agent should be guided primarily by the relative solubility of the penetrating agent in this substance.

In a previous investigation (1) a technique for determining the ability of an ointment material to resist penetration by diffusion was described. In the present study this technique has been employed to make possible a careful evaluation of several factors which are of importance in determining protective capacity. Further, since vapors are often penetrating forms, a technique

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for following penetration of vapors was developed.

EXPERIMENTAL

Apparatus.—The apparatus employed in these studies has been described in a previous paper (1).

Procedure.—In addition to liquid-contact penetration studies which have been described previously (1), vapor phase penetration was also studied in this investigation. For this case, a volatile penetrant was selected as a model and its penetration from solutions in nonvolatile solvents was studied. The mechanism of penetration was identical with that for liquid-contact type of penetration except for an initial delay period during which the penetrant in solution equilibrated with its vapor. The penetrating agent then transferred to the barrier as a vapor.

In the procedure employed, exactly 1.0 or 1.5 ml. of a solution containing a known concentration of penetrating agent in a nonvolatile solvent was pipetted into one of the chambers of the barrier cell apparatus. In the same manner 1.0 or 1.5 ml. of solvent was placed in the opposite chamber. The assembly was stoppered and placed in a 25° constant-temperature bath where it was subjected to mild, continuous shaking. At periodic intervals approximately 0.1-Gm. samples were withdrawn[1] from each of the chambers and accurately weighed. The amount of penetrant in each sample was determined spectrophotometrically using ethanol as the solvent.

The distribution coefficients for the partitioning of nitrobenzene between white petrolatum and the various ethanol solutions were determined by a direct contact method. A modification of the countercurrent extraction scheme of Craig and Craig (2) was employed wherein nitrobenzene was first partitioned into the white petrolatum and then allowed to redistribute between the white petrolatum and fresh solvent. At equilibrium the nitrobenzene content of the solvent was determined in both cases. Since the same volume of hydroalcoholic solution was used in both equilibrations, the distribution coefficient (DC) was calculated from the ratio of the nitrobenzene concentrations according to the equation

$$DC = 1/r(R - 1)$$

where r = the ratio of the volumes of the white petrolatum and hydroalcoholic media, and R = the ratio of nitrobenzene concentrations in the hydroalcoholic media after the first and second equilibrations.

Distribution coefficients for nitrobenzene between nonvolatile liquids and semisolids were determined by means of an isopiestic procedure. In this way it was possible to determine the distribution coefficient between both miscible and immiscible materials.

Distribution coefficients determined by either of the above methods were felt to be satisfactory for the purpose of this work.

[1] In evaluating the experimental data a correction factor was applied to account for the change in volume due to removal of sample.

RESULTS AND DISCUSSION

Nitrobenzene was selected as the model penetrating agent because it possessed the desired volatility and solubility acquirements. Barrier materials selected were white petrolatum, Jelene,[2] Kel F 150,[3] combinations of cerese yellow wax with heavy liquid petrolatum and polyethylene glycol 400 monostearate. Solvents employed for the nitrobenzene were *n*-amyl sebacate, heavy liquid petrolatum, and silicone DC 200 fluid, 1,000 c. p. s.[4] For liquid-contact penetration studies solutions of ethanol in water at various concentrations were employed as solvents for the nitrobenzene.

Permeability constants for the penetration of barrier materials by vapor phase penetration were determined by graphic solution of the equation

$$\log (C_o - 2C_B) = (-2K/2.303)t + \log C_o \quad \text{(Eq. 1)}$$

where C_o is the initial concentration of penetrant in the solvent on the side of origin, C_B is the concentration of penetrant in the receiving chamber, t is the time of sampling, and K is the permeability constant. The relationship between the permeability constant and the cross-sectional area of the barrier A, the diffusion coefficient D, the distribution coefficient DC, the volume of solution on each side of the barrier V, and the thickness of the barrier L is given by the equation

$$K = AD(DC)/VL \quad \text{(Eq. 2)}$$

In Table I the permeability constants obtained for the penetration of various barriers by nitrobenzene vapor are listed along with the solvent employed and the thickness of the ointment film for each determination. The distribution coefficient also given in the table is defined as the ratio of the concentration of the nitrobenzene in the surface layer of the ointment film to its concentration in the solvent employed. Similar data for the liquid-contact permeation of white petrolatum by nitrobenzene from various ethanol-water solutions are also given in this table.

In Fig. 1 the relationship between the permeability constant and the distribution coefficient for nitrobenzene vapor penetration of white petrolatum barriers is shown graphically. Similar data for liquid-contact permeation studies are plotted in Fig. 2. As expected from the relationship shown by Eq. 2, K is seen to vary directly with the distribution coefficient.

The significance of this relationship is that the optimum base to be selected for a protective ointment is one in which the penetrating agent is poorly soluble. In such a situation the concentration gradient of penetrant in the barrier, which is the driving force for the diffusion of this agent across the barrier, is minimal since the partition coefficient of the penetrating agent would not favor the barrier material.

This is borne out by the findings for the penetration of the fluorocarbon polymer film where K is found to be approximately one-tenth that for the penetration of Jelene. Due to the nature of the fluorocarbon polymers nitrobenzene is poorly soluble

[2] Research Products Corp. (Squibb Plastibase).
[3] Fluorocarbon wax, M. W. Kellogg Co.
[4] Dow Corning.

TABLE I[a]

Barrier Material	Solvent	Distribution Coefficient	Thickness (cm.)	Permeability Constant × 10^4
White petrolatum	*n*-amyl sebecate	0.21	0.3213	1.0
White petrolatum	*n*-amyl sebecate	0.21	0.2504	1.2
White petrolatum	*n*-amyl sebecate	0.21	0.1798	1.7
White petrolatum	*n*-amyl sebecate	0.21	0.1245	2.4
White petrolatum	*n*-amyl sebecate	0.21	0.0833	3.1
White petrolatum	*n*-amyl sebecate	0.21	0.0434	5.2
Jelene	*n*-amyl sebecate	...	0.0434	7.3
Kel F 150	*n*-amyl sebecate	0.06	0.0434	0.7
Cerese yellow wax, 20% Heavy liquid petrolatum, 80%	*n*-amyl sebecate	...	0.0434	4.4
Cerese yellow wax, 40% Heavy liquid petrolatum, 60%	*n*-amyl sebecate	...	0.0434	2.5
Polyethylene glycol 400 monostearate	*n*-amyl sebecate	...	0.0434	3.6
White petrolatum	silicone DC 200 Fluid, 1,000 c. p. s.	1.8	0.0434	35.3
White petrolatum	heavy liquid petrolatum	1.0	0.0434	16.5
White petrolatum	ethanol-water 51.4% w/w	1.3	0.0434	4.5
White petrolatum	ethanol-water 33.1% w/w	4.9	0.0434	16.5
White petrolatum	ethanol-water 16.1% w/w	15.2	0.0434	62.5
White petrolatum	ethanol-water 40% w/w	30.2	0.0434	100

[a] The data given in the table represent the average of a minimum of two, and usually three to five determinations.

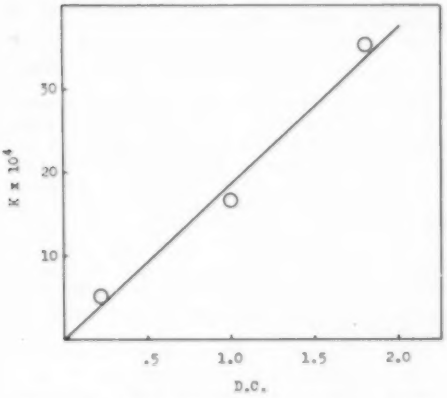

Fig. 1.—Relationship between the permeability constant and the distribution coefficient (vapor phase permeation).

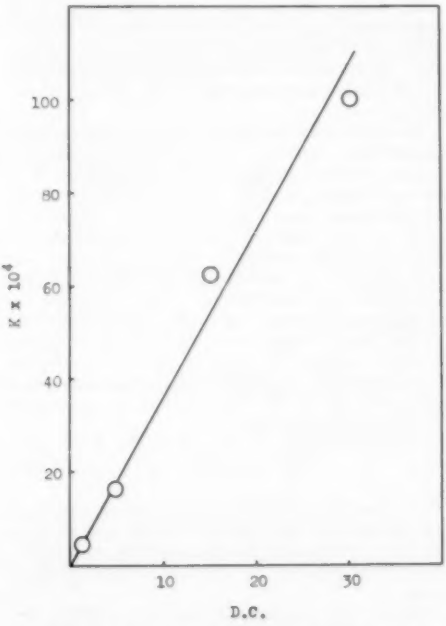

Fig. 2.—Relationship between the permeability constant and the distribution coefficient (liquid-contact permeation).

in it, thus we find that the distribution coefficient for nitrobenzene between Kel F 150 and *n*-amyl sebacate is 0.06.

It is interesting to note the effect of the addition of cerese yellow wax to heavy liquid petrolatum on the permeability constant. It may be that the observed decrease in the permeability constant is due to crystallization of the wax in the heavy liquid petrolatum. If this were to occur, the nitrobenzene molecules which partition into the wax would be expected to show a much lower rate of diffusion than those molecules passing through the liquid petrolatum portion of the mixture. If the partition favored the wax, a significant decrease in the rate of permeation could be expected.

According to Eq. 2 the permeability constant may also be expected to be an inverse function of the thickness of the ointment film through which the penetrating agent must diffuse. Before this can be evaluated, however, it is necessary to determine the influence of the apparatus and related phenomena on the experimentally determined permeability constants. This is accomplished by plotting

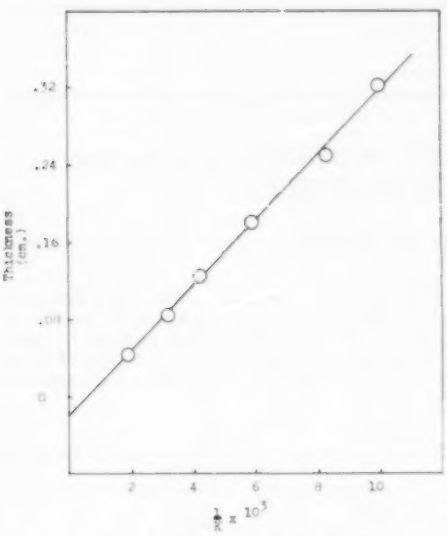

Fig. 3.—Determination of correction factor for the apparatus and related phenomena.

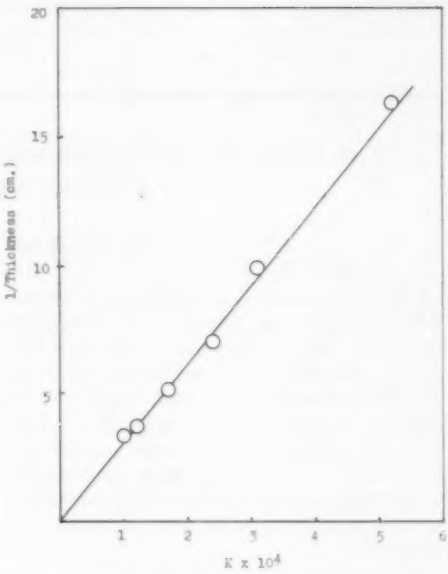

Fig. 4.—Inverse relationship between the permeability constant and barrier thickness.

the reciprocal of the permeability constant as a function of barrier thickness. This plot is shown in Fig. 3. Extrapolation of the plot to the y-axis gives an intercept of -0.018 cm. Thus the hold-up of the apparatus and related phenomena in the process is equivalent to a white petrolatum barrier 0.018 cm. thick.

Using this correction, the relationship between K and L predicated by Eq. 2 can be expressed graphically as shown in Fig. 4.

It is evident from the plot that satisfactory linear agreement exists between the permeability rate and inverse of the barrier thickness. Moreover the line passes definitely through the origin, as expected.

The present findings constitute further proof of the validity of the experimental procedures proposed for determining permeability of protective films to both liquid and vaporous penetrants. The theoretical relationship among such several factors as partition coefficients, diffusion rate, barrier thickness, etc., appear to be well maintained, provided suitable corrections are introduced to provide for deviations introduced by the barrier supports and other partial obstacles to free diffusion.

A practical significance can be attached to this relationship with regard to the use of protective ointments. When a penetrating agent is able to traverse the cells of the stratum corneum only slowly, the protection afforded by a protective ointment will be related to the depth to which the ointment fills the cracks and pores in the cornified layer of the skin. Thus, in such a case the protective cream should be formulated so that among other factors its viscosity and flow properties allow maximum depth of penetration of the stratum corneum by the protective base.

SUMMARY

A method has been described for studying the permeation of protective ointment films by penetrating agents in the vapor state. Relationships established follow theoretical predictions.

It has been shown that the permeability constant for the penetration of an ointment film is related to the partition coefficient in a linear fashion. Thus maximum protection against a given penetrating agent would be afforded by an ointment film in which the penetrating agent is poorly soluble.

It has also been verified that the thickness of an ointment film bears an inverse relationship to the permeation constant.

The procedures employed in this study may be adapted to the study of protective ointments for a wide variety of chemical penetrating agents.

REFERENCES

(1) Lueck, L. M., Wurster, D. E., Higuchi, T., Lemberger, A. P., and Busse, L. W., THIS JOURNAL, **46**, 694(1957).

(2) Weissberger, "Technique of Organic Chemistry," Vol. III, Interscience Publishers, Inc., New York (1950).

Notes

A Note on the Psychomotor Effects of Diphenhydramine Hydrochloride and Dimenhydrinate*

By RICHARD G. PEARSON†

ALTHOUGH SEVERAL motion-sickness preventives have been found to give satisfactory protection without immoderate subjective complaints, the effects of these preparations have not been evaluated over a wide range of human abilities. As part of a broad program concerned with the effects of drugs upon psychological processes (1, 2), the present study was instigated to determine the effects of two of the more popular motion-sickness preventives upon perceptual-motor skill.

METHOD

A total of 48 volunteer basic Air Force trainees served as subjects. The tracking task was provided by the USAF SAM Multidimensional Pursuit Test, Model CM 813E (3). This task requires subjects to monitor the apparently random movements of four instrument pointers from their null positions, and to compensate accordingly by coordinated adjustments of throttle, stick, and rudder controls. When all four pointers are centered concurrently, a timer cumulates a subject's proficiency score. Task trials are one minute in length, separated by rest periods of fifteen seconds.

The drug preparations consisted of diphenhydramine hydrochloride (50 mg. Benadryl®), dimenhydrinate (100 mg. Dramamine®), and placebo (lactose), and were administered orally in No. 1 pink capsules.

The procedure can be divided appropriately into three parts: (*a*) practice on the task for 40 trials in order for subjects to establish a substantial level of skill; (*b*) a ten-minute rest interval during which the drug capsule was administered and appropriate motivational instructions were presented; and (*c*) post-treatment task performance of four hours. During both pre-treatment and post-treatment periods the subject's performance for each cycle of work (eight trials) was recorded. A randomized schedule was followed with two subjects being tested per day until each treatment group contained 16 subjects.

RESULTS

To determine the effects of the pharmacologic treatments on terminal performance, the average proficiency levels attained for the final five cycles of practice were analyzed by the method of covariance using as the independent variable the mean performance of the last three pre-treatment cycles. The results shown in Table I indicate that significant differences were produced by the experimental treatments. Further analysis by means of *t*-tests performed on the adjusted treatment means reveals both diphenhydramine hydrochloride (mean = 85) and dimenhydrinate (mean = 94) groups to be significantly inferior to the placebo group (mean = 141), during terminal performance; at the same time the two experimental groups are not significantly different from one another. The picture becomes clearer when one refers to Fig. 1 which plots the average course of performance throughout the post-treatment period. The deleterious effect upon performance of both diphenhydramine hydrochloride and dimenhydrinate is well established

TABLE I.—ANALYSIS OF VARIANCE OF TERMINAL PERFORMANCE

Source of Variance	*df*	Mean Square	F	P
Drugs	2	14082.96	3.60	.04
Error	44[a]	3917.22		
Total	46			

[a] Reduced 1 *df* for regression.

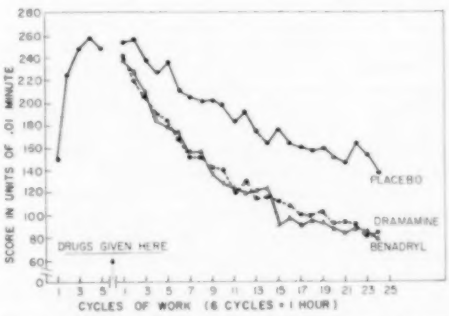

Fig. 1.—Effects of pharmacologic treatments upon task proficiency.

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early in the post-test, and this effect becomes more pronounced throughout the task. Of additional interest is the indistinguishability of these two curves as they parallel each other in their downward course. However, it might be expected that the action of diphenhydramine hydrochloride and dimenhydrinate in this case would be similar, since the two drugs have a close chemical similarity. The latter drug is actually a combination of diphenhydramine and 8-chlorotheophylline. The chlorotheophylline moiety is known to contribute nothing to the therapeutic action of dimenhydrinate, and, at least as far as the present study is concerned, it evidently has no secondary action. Thus in actuality what has been observed in the present study are the effects upon performance of two almost equal doses of diphenhydramine.

It is to be concluded that, *relative to competitive preparations*, the effects of diphenhydramine hydrochloride and dimenhydrinate in this study are such that one could hardly recommend them for motion-sickness prophylaxis in situations wherein perceptual-motor skill is required of the individual. There are, of course, other characteristics of drugs which one may wish to consider in any final evaluation.

REFERENCES

(1) Payne, R. B., *Int. Rec. Med.*, **168**, 32(1955).
(2) Payne, R. B., and Moore, E. W., *J. Pharmacol. Exptl. Therap.*, **115**, 480(1955).
(3) "Apparatus Tests" (AAF Aviat. Psychol. Program Res. Rep. No. 4 Restricted Supplement) edited by A. W. Melton, U. S. Government Printing Office, Washington, 1947.

A Note on the *In Vitro* Inhibition of *Endamoeba histolytica* by Diazobarbituric Anhydride*

By MITSURU NAKAMURA

DURING THE STUDIES on the effect of antimetabolites on the growth of *Endamoeba histolytica*, diazobarbituric anhydride[1] was found to be a potent inhibitor of growth of this protozoan. This compound [5,7–dihydroxy–(1,2,3)–oxadiazolo–(5,4,*d*)–pyrimidine] was of particular interest since Nakamura and Baker (1) reported that *E. histolytica* requires purines and pyrimidines for growth in the absence of associated bacteria.

EXPERIMENTAL

The assay of the amebacidal activity of the compound was performed according to methods described earlier (2–5). Various concentrations of the diazobarbituric anhydride were tested in a medium consisting of egg slants overlaid with serum and Ringer solution. Adenosinetriphosphate and ribose–5–phosphate were added, since they are essential growth factors for the amebas. Penicillin and streptomycin were also added to kill the bacteria that were introduced with the amebic inoculum. Therefore, the amebacidal end points determined in these experiments are due to direct amebacidal effects.

Diazaobarbituric anhydride possessed an amebacidal end point of 1/100,000 (0.001 mg./ml.). This agent was amebastatic at concentrations of 1 mg./ml. (1/1,000). Reversal of this amebic inhibition was possible with adenylic acid (5 μg./ml.), guanylic acid (10 μg./ml.), cytidylic acid (1 μg./ml.), thymidylic acid (50 μg./ml.), and uridylic acid (100 μg./ml.). The inhibitory activity of the diazobarbituric anhydride could not be reversed by adenine, guanine, cytosine, uracil, thymine, adenosine, guanosine, and 4–amino–5–imidazole–carboxamide.

It would appear from these studies that diazobarbituric anhydride exerts an antimetabolite effect on *E. histolytica*. These experiments, however, do not indicate whether the inhibition is competitive or noncompetitive. Since the inhibition was reversed by the nucleotides and not by the free purine and pyrimidine bases and nucleosides, it would seem that the compound blocks metabolism at a step prior to the synthesis of the nucleotides. DiCarlo, *et al.* (6), reported that in the yeast, *Torula utilis* and *Saccharomyces cerevisiae* Hansen, diazobarbituric anhydride antagonizes the assimilation of cytosine, possibly by inhibition of cytosine deaminase.

REFERENCES

(1) Nakamura, M., and Baker, E. E., *Am. J. Hyg.*, **64**, 12(1956).
(2) Nakamura, M., and Baker, E. E., *Proc. Soc. Exptl. Biol. Med.*, **92**, 723(1956).
(3) Nakamura, M., *Nature*, **178**, 1119(1956).
(4) Nakamura, M., *Experientia*, **13**, 29(1957).
(5) Nakamura, M., and Jonsson, S., *Arch. Biochem. and Biophys.*, **66**, 183(1957).
(6) DiCarlo, F. J., Schultz, A. S., and Kent, A. M., *J. Biol. Chem.*, **194**, 769(1952).

* Received January 25, 1957 from the Department of Bacteriology, Montana State University, Missoula.
[1] Obtained through the courtesy of Dr. Frederick J. DiCarlo, Biochemistry Division, The Fleischmann Laboratories, Stamford, Conn.

A Note on the Volatile Oil from the Roots of *Pteryxia terebinthina* (Hook.) Coult. and Rose Var. *Terebinthina**

By TRACEY G. CALL† and EARL B. FISCHER

AN EXHAUSTIVE SEARCH of the literature has failed to reveal any previous chemical studies of *Pteryxia terebinthina* (Hook.) Coult. and Rose var. *terebinthina*, a xerophyte growing in north central and eastern Oregon, south and east central Washington, and perhaps in adjacent Idaho.

In connection with other studies carried out on this species, the volatile oil was isolated and a condensation of these findings is presented here.

The roots were collected in Sherman County, Ore., and the identification of herbarium specimens was made by Dr. Lincoln Constance of the University of California at Berkeley, Calif. The roots were broken into small pieces and air dried. Several lots collected at various seasons and with various moisture contents were milled to a #20-mesh fineness and steam distilled. The oil yield of most lots ranged from 4.41 to 8.32 per cent on an air-dry basis. The colorless-to-pale-yellow oils were dried with anhydrous sodium sulfate and the physical constants were determined as follows: specific gravity at 25°/25°, range 0.8181 to 0.8810, mostly about 0.8560; optical rotation at 25°, range from +5.4° to +18.2°; refractive index at 20°, range 1.4721 to 1.4783, mostly about 1.4736.

Most of the samples were soluble in 1.0 to 1.5 volumes of 95 per cent alcohol and in 4.0 to 4.5 volumes of 90 per cent alcohol. All were insoluble in 10 volumes of 85 per cent alcohol.

Fractionation of 165 ml. of oil at a pressure of 3 to 4 mm. of mercury yielded 130 ml. of oil in the 39° to 43° range with the following physical constants: specific gravity at 25°/25°—0.8772; optical rotation at 25°—plus 16.7°; refractive index at 20°—1.4727; boiling point at 677 mm. of mercury—168–170°.

* Received February 11, 1957, from the School of Pharmacy, Montana State University, Missoula, and the College of Pharmacy, University of Minnesota, Minneapolis.

† Present address: Sunkist Growers, Research Dept., Corona, Calif.

Book Notices

New and Nonofficial Remedies 1957. Evaluated by the Council on Pharmacy and Chemistry. J. B. Lippincott Company, 1957. xxv + 582 pp. 12.5 x 19.5 cm. Price $3.35.

This exceptionally valuable publication of the American Medical Association contains descriptions of drugs evaluated by the Council on Pharmacy and Chemistry since the compilation of the 1956 edition. The new policy of the A. M. A. has been reflected in the 1956 and 1957 editions by the term "Evaluated" which has replaced "Accepted" by the Council and in the shorter period between appearance of drugs and their evaluation. The N. N. R. monographs continue to be models of concise presentation of all the important known information relating to new drugs and, when reported and evaluated, add new information to descriptions of drugs retained from earlier editions.

Morphine & Allied Drugs. By A. K. REYNOLDS and LOWELL O. RANDALL. University of Toronto Press, Toronto, Canada, 1957. xiii + 393 pp. 15 x 23 cm. Price $10.

This book attempts, rather successfully, to correlate information on the potent analgesics now available. The material is presented under the general topics of morphine, its natural and semisynthetic derivatives, and the important new synthetic analgesics. The text material is divided under the following headings: Alkaloids of opium, Other papaveraceous alkaloids, Synthetic analgesics, Antagonists to analgesics and theories relating chemical structure to analgesic activity. A brief summarizing chapter concludes the text, and a general index is appended. The text stresses physiological actions with brief references to the chemistry of the compounds discussed. A bibliography of 1400 references is given for Parts I and II of the text and the other sections have individual bibliographies. The chapter on the relation of chemical structure to analgesic activity includes useful structural formulas. The book is a monograph that should be available in pharmacy and medical libraries to students and advanced investigators.

Expectant Motherhood. 3rd ed., revised. By NICHOLSON J. EASTMAN. Little, Brown & Company, Boston, 1957. xiv + 198 pp. 12.5 x 19 cm. Price $1.75.

This pocket-size book is intended as a supplementary guide through pregnancy. The author is a Professor of Obstetrics and is Obstetrician-in-Chief to the Johns Hopkins Hospital.

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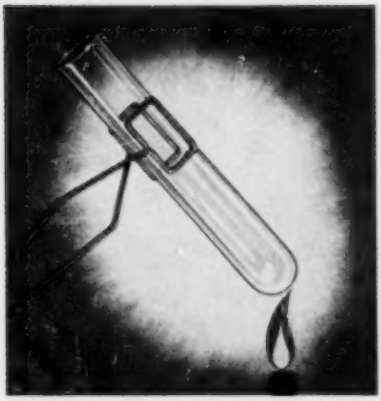

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